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Biochemical Changes in Source and Sink of Chickpea Influenced by Foliar Application of Ethrel, Kinetin and Boron

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ABSTRACT

Worldwide among the pulse crops, the production of chickpea is one of most important thrust research area needs to be addressed seriously.The present investigation focused on biochemical changes in chickpea (kak 2 variety) source and sink with foliar applications of two plant growth regulators (Ethrel, Kinetin) and one micronutrient (Boron), applied in concentrations of 250 ppm,10 ppm and 0.25% respectively, alone and in combinations. Field experiment was conducted during rabi 2013-14 at college form, agricultural college bapatla, Andhra pradesh in clay loam soils in randomized block design with eight treatments in three replications. Chickpea highly responded to foliar applications and there was significant changes in source biochemical changes viz., relative water content (RWC), photosynthetic pigments (chl a, b, carotenoid and total chlorophyll), nitrate reductase activity (NR) and sink biochemical changes viz., total soluble protein, total starch, iron and zinc content. The results of experiment indicated that RWC significantly increased 16.5, 13.1 and 12.2 percent with Ethrel, Kinetin and Ethrel + Kinetin sprays respectively at 45 DAS compared to control. Chlorophylls and carotenoids are important pigments involved in photosynthetic activity. These levels in chickpea leaves increased up to 45 DAS and later decreased due to leaf senescence. At 45 DAS, all photosynthetic pigment contents were observed maximum with 10 ppm Kinetin at 35DAS,followed by the sprays of Ethrel at 25 DAS + Kinetin at 35 DAS and Ethrel at 25 DAS. Maximum increase in Nitrate reductase activity (NRA) was observed in Kinetin sprayed plants. The protein content of seed recorded maximum in combined foliar sprays of Ethrel, Kinetin and Boron (T_7), which was 17 percent higher than that in seeds of control plants. The highest value of starch content was recorded with spray of 0.25% Boron at 45 DAS (T_3) followed by the sprays of Kinetin + Boron (T_5) and Ethrel + Kinetin + Boron (T_7). Increase in Fe and Zn content in seeds was observed with spray of 0.25% Boron at 45 DAS (T_3). In conclusion, present review indicates that the pattern of response of both growth hormones and micronutrients were greatly influenced the biochemical factors in chickpea source and sink.

Key words: *Plant growth regulators, Nutrients, Chickpea leaf and Seed and Quality characteristics*

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INTRODUCTION

A pulse, including chickpea (*Cicer arietinum* L) is one of the most important crops of the world due to their nutritional quality. It remarkably predominates among other pulse crops in terms of both area and production. All over the world, chickpea occupies an area of 139.81 m ha with a production of 137.3 m t and productivity range is around 986 kg ha^{-1} [10]. Chickpea seeds are rich source of protein (24.63 %), fat (5.62 %), carbohydrates (64.60 %), fiber (1.85 %) and minerals like Fe (6.85), Zn (3.83 mg /100 mg).Chickpea seeds are usually consumed at the raw green tender stage (unripe stage) or in the form of mature dry seeds after parching as a popular snack food[1]. Because now a days Fe and Zn deficiencies are reportedly the most widespread and commonly observed micronutrient deficiencies in human beings. And also these play a crucial role in growth and development of human. Despite its importance, few studies have been conducted to analyse the effect of application of Plant growth regulators (PGR's) and micronutrients on chickpea to increase the quality parameters (protein, starch, Fe and Zn) in seed.

Plant growth regulators (promoters, inhibitors or retardants) play a key role in contributing internal mechanisms of plant growth by interacting with key metabolic processes such as, nucleic acid metabolism and protein synthesis. Growth retardants are also known to reduce internodal distance, thereby enhancing source-sink relationship and stimulate the translocation of photo-assimilates to the seeds. The enhanced source-sink relationship with the use of plant growth regulators stimulate the translocation of photo assimilates, thereby increasing the productivity. The above statements were concurrent with the findings of Upadhyay, R. G.[39], in Chickpea.

On other hand, adequate and balance supply of plant nutrients is a pre requisite in increasing legume yield through their effects on the plant itself, on the nitrogen-fixing symbiotic process and the effective use of the major and secondary Nutrients, resulting changes in biochemical levels and yields. The magnitude of yield losses due to nutrient deficiency also varies among the nutrients[2]. Supplementary foliar fertilization during crop growth can improve the mineral status of plants and increase the crop yield by absorbing nutrients through the leaves remarkably rapid and nearly complete[9]. Hence, this study is conducted to investigate the influence of foliar application of Ethrel, kinetin and boron on biochemical characteristics of chickpea in leaves and seeds.

RWC (It is a measure of water deficit in the leaf) of leaves is very important factor in chickpea increased yield. RWC was introduced as a best criterion for plant water status which, afterwards was used instead of plant water potential as RWC referring to its relation with cell volume, accurately can indicate the balance between absorbed water by plant and consumed through transpiration[14]. This influences the ability of the plant to recover from stress and consequently affects yield and yield stability. Generally, it seems that osmoregulation is one of the main mechanisms preserving turgor pressure in most plant species against water loss from so, it causes plant to continue water absorption and retain metabolic activities[19].The present study to estimate RWC of chickpea leaves by external application of Ethrel, kinetin and boron.

Plant pigments Chlorophyll a, chlorophyll b and carotenoids are main photosynthetic pigments, and they are indicators of photosynthetic capability of plant tissues[26]. Net photosynthesis and stomatal conductance are significantly affected due to changes in chlorophyll content and chlorophyll fluorescence, damage of photosynthetic apparatus and chloroplast structure[8]. The reduction in chlorophyll and other pigments contents may reduce carbon fixation that eventually supply energy and substrates for metabolic pathways. This finally may cause reduction in plant growth, development and yield[40]. There is little information available on the external application of PGR's and nutrients

may increase the chlorophyll content in groundnut[34].

Nitrate reductase activity (NRA) provides a good estimate of the nitrogen status of legume plants by regulatory role in the NO_3^{2-} assimilation[16,13] and is correlated with growth and plant yield[36]. NR is a most important enzyme and it is to be exploited the activities of key enzymes in the assimilatory pathway[24]. Reductions in NR activity, reduced nitrogen fixation and ammonia assimilation in nodules have been reported in legumes by Balestrasse *et al.*[4]. The reduction of NRA in both leaves and roots was also proportional to the reduction in root and shoot dry mass accumulation and source to sink assimilate transport in chickpea. According to Fatima *et al.*[11], all these activities in chickpea generally triggered by plant growth hormones and nutrients. So, the present study attempts to investigate the effects of growth regulators and nutrients on the Nitrate reductase activity (NRA) of chickpea leaves.

MATERIAL AND METHODS

The field experiment was conducted during the *Rabi* season of 2013-14 at Agricultural College Farm, Bapatla to study the effect of plant growth regulators Ethrel, kinetin and micro nutrient boron on productivity of chickpea with eight treatments and three replications in a randomized block design. The soil of experiment field is black clay loam having P^H-7.8, EC-0.29 dSm^{-1},organic carbon 5.1g kg^{-1},198 kg nitrogen ha^{-1}, 33.0 kg P_2O_5 ha^{-1} and 821 kg K_2O ha^{-1}.The seeds of chickpea variety kak 2 were sown by dibbling at spacing 30×10 cm. The recommended package of practices (except treatments) was followed to raise the crop. Foliar sprays of growth regulators Ethrel and Kinetin and micronutrient Boron were given to the crop in the following manner.T_1 - Ethrel @ 250 ppm at 25DAS, T_2 - Kinetin @ 10 ppm at 35DAS, T_3 - Boron @ 0.25% at 45DAS, T_4 - Ethrel @ 250 ppm at 25DAS + Kinetin @ 10 ppm at 35DAS, T_5 - Kinetin @ 10 ppm at 35DAS + Boron @ 0.25% at 45DA,T_6 - Ethrel @ 250 ppm at 25DAS + Boron @ 0.25% at 45DAS, T_7 - Ethrel @ 250 ppm at 25DAS + Kinetin @ 10 ppm at 35DAS + Boron @ 0.25% at 45DAS,T_8 - control (No spray).All biochemical parameters in source were analysed at four stages viz. 15 DAS, 30 DAS, 45 DAS and 60 DAS and those in sink were assayed at once after harvest by using standard protocols of various scientists. Statistical analysis of the individual data of various characters studied in the experiment was carried out using standard statistical procedures as described by Panse and Sukhatme[28]. Standard error of mean, critical difference (C.D.) at 5 per cent level of probability and coefficient of variance were worked out for the interpretation of the results.

BIOCHEMICAL PARAMETERS IN SOURCE:

Relative Water Content (%):

The relative water content (RWC%) of leaf samples collected from different treatments was determined by following the method described by Slatyer and Mcilroy[35]. Detached leaf samples were weighed immediately to record the fresh weight and then floated on distilled water for 4 hours to obtained turgid weight.The turgid weight was recorded after blotting the excess water on the surface of the sample. Dry weight was recorded after drying the samples in oven at 80ºC for two days till constant weight occurred. The relative water content of leaves was calculated by substituting recorded values in the following equation:

$$\text{Relative Water Content \%} = \frac{(\text{Fresh weight - Oven Dry Weight})}{(\text{Saturated Weight- Oven Dry Weight})} \times 100$$

Chlorophyll Content in Leaves (mg g^{-1} fresh weight)

Chlorophyll content in leaves was estimated colorimetrically by dimethyl sulphoxide (DMSO) method as described by Hiscox and Stam[21]. Finely chopped 300 mg chickpea leaves were weighed and taken in stoppered test tubes. 10 ml of DMSO was added to each tube and incubated at 60-65^0 C for 3 hours. After incubation, the tubes were allowed to cool at room temperature and the volume was made up to a total of 10 ml by adding DMSO. The optical density (OD) was recorded at 480, 510, 645, 652 and 663nm separately using Systronics Spectrophotometer (Model 105) and total chlorophyll, chlorophyll a, chlorophyll b and carotenoids were calculated by using the standard formulae of Arnon[3].

Estimation of Nitrate Reductase Activity:

Nitrate reductase activity in the leaf was estimated using the method suggested by Hageman and Flesher[20]. The nitrite formed was estimated by the method described by Nicholas *et al*.[27], by measuring the absorbance of the pink colour at 540 nm using spectrophotometer and the enzyme activity was expressed as µmol NO_2 g^{-1} of fresh weight hr^{-1}($\mu M NO_2^- g^{-1} hr^{-1}$).

BIOCHEMICAL PARAMETERS IN SINK:

Seed protein content (mg g^{-1} dry weight)

After harvesting, the pods were threshed and the seeds were collected. These seeds were used to estimate soluble protein content of chickpea kak2 variety. soluble protein (mg g^{-1}d.wt) content in the seed sample was estimated by Folin-Ciocalteau reagent method Lowry *et al*.[23]. 500mg of seed sample was ground well with a pestle and mortor using 5-10 ml of potassium sodium tartarate buffer, subjected to centrifugation and the supernatant was collected.0.2ml of extract was taken volume made to 1 ml by adding distilled water and allowed to stand for 10minutes. Folin-Ciocalteau reagent (0.5 ml) was added and incubated in dark room temperature for 30 minutes. The intensity of blue col our developed was measured at 660 nm. Blank was prepared without plant sample and the absorbancewas measured.Protein content in the sample was estimated using blank value and the standard curve prepared with bovine serum albumin.

Starch Content:

Starch content in seeds was estimated by Anthrone Reagent method[33]. 0.5 g of seed sample was homogenized, centrifuged in hot 80% ethanol to remove sugars. Then the residu retained was washed repeatedly with hot 80% ethanol till the washings do not give green colour with anthrone reagent. Then, the residue was dried well over a water bath, 5ml of water and 6.5 ml of 52% perchloric acid were added, centrifuged at 0^0 C for 20 minutes and the supernatants were pooled repeatedly using fresh perchloric acid and made upto 100 ml. 0.1 ml of the supernatant was made upto 1 ml with distilled water, 4 ml of anthrone reagent was added, heated in a boiling water bath for eight minutes, cooled rapidly and the intensity of green colour was read in spectrophotometer at 630 nm. Standard curve was prepared with standard glucose solution. Glucose content in sample was obtained from the standard graph and multiplied by the factor 0.9 to arrive the starch content.

Fe and Zn content :

The seeds were dried in a ventilated oven for approximately 78 h at 60ºC to a constant weight and then ground, these seed samples were digested in a nitric-perchloric acid mixture (9:4) and analyzed for Fe and Zn with Atomic Absorption Spectrometer model Hitachi 170-30, using appropriate cathode lamps[22]. The content was estimated and expressed in µg g^{-1}.

RESULTS AND DISCUSSION

BIOCHEMICAL PARAMETERS:

Relative Water Content (%)

The influence of Ethrel, Kinetin and Boron sprays on Relative Water Content (RWC) of chickpea leaves was significant at 30DAS and onwards (Table 1).

There was significant difference in RWC at 30 DAS with the application of Ethrel, varied from 63.4 to 75.7% with an average of 69.5% and it was found higher than

control (58.7%). At 45 DAS plants treated with Ethrel recorded significantly higher RWC followed by Kinetin and Ethrel + Kinetin than control. The mean of increase in RWC from 30-45 DAS was 7.9% with Ethrel spray, 9.4% with Kinetin spray and 3.7% with both Ethrel and Kinetin spray.

At 60 DAS, spray of 0.25% Boron at 45 DAS (T_3- 56.6%) recorded significantly higher RWC, followed by the spray of 250 ppm Ethrel at 25 DAS +0.25% Boron at 45 DAS (T_6 – 55.5%) and 250 ppm Ethrel at 25DAS + 10 ppm Kinetin at 35DAS + 0.25% Boron at 45DAS (T_7 -55.3%) compared to control (T_8 -43.9%). The remaining treatments T_7, T_2, T_4 and T_5 was on par with control.

Relative water content is a measure of the water status of a plant. In the present investigation, the higher values of RWC were observed at 45 DAS. Compared to control RWC of leaves was 16.5, 13.1 and 12.2 percent higher with Ethrel, Kinetin and Ethrel + Kinetin sprays respectively. At 60 DAS the spray of Boron at 45 DAS alone and in combination of Ethrel and Ethrel + Kinetin maintained 26.0 to 28.9 per cent higher RWC in leaves than control. This might be due to the role of Kinetin in reducing leaf senescence, maintaining metabolic activities consequently more absorption of water and nutrients from the soil, which reflected on growth as reported by Sadak *et al.*[32].

Table 1: Effect of foliar sprays of Ethrel, Kinetin and Boron on Relative Water Content (%) of Chickpea leaves

Treatments	Relative Water Content (%)			
	Days After Sowing			
	15	**30**	**45**	**60**
T_1: Ethrel @ 250 ppm at 25 DAS	63.0	63.4	70.2	52.9
T_2: Kinetin @ 10 ppm at 35 DAS	58.4	60.9	73.5	44.1
T_3: Boron @ 0.25% at 45 DAS	76.8	82.8	84.8	56.6
T_4: Ethrel @ 250 ppm at 25DAS + Kinetin @ 10 ppm at 35DAS	66.5	67.7	71.4	46.6
T_5: Kinetin @ 10 ppm at 35DAS + Boron @ 0.25% at 45DAS	66.1	68.3	74.5	54.4
T_6: Ethrel @ 250 ppm at 25DAS + Boron @ 0.25% at 45DAS	75.5	75.7	84.6	55.5
T_7: Ethrel @ 250 ppm at 25DAS + Kinetin @ 10 ppm at 35DAS + Boron @ 0.25% at 45DAS	70.7	71.1	74.8	55.3
T_8: Control	56.7	58.7	60.9	44.0
SEm + **–**	**4.8**	**4.7**	**4.4**	**3.6**
CD (P= 0.05)	**NS**	**14.2**	**13.2**	**11.0**
CV (%)	**12.4**	**11.8**	**10.2**	**12.3**

Photosynthetic Pigments:

Chlorophyll 'a':

Significant differences in chlorophyll 'a' content was found due to the Ethrel, Kinetin and Boron sprays at all stages (Table 2.1). At 30 DAS, chlorophyll 'a'content with Ethrel spray varied from 0.89 to 0.91 mg g^{-1} with an average of 0.90 mg g^{-1} and it was on par with control (0.88 mg g^{-1}).

At 45 and 60 DAS, there was significant increase in chlorophyll 'a'content in all treatments over control. Among the treatments, 10 ppm Kinetin at 35 DAS (T_2) showed higher chlorophyll 'a' (1.46& 1.13 mg g^{-1}respectively) compared to control and it was followed byT_4-250 ppm Ethrel at 25DAS + 10 ppm Kinetin at 35DAS (1.37& 1.05 mg g^{-1}respectively) and T_1-250 ppm Ethrel at 25DAS (1.36 & 0.95 mg g^{-1}respectively). The effect of treatments T_7, T_5 ,T_6 and T_3 on chlorophyll 'a'content was on par with control.

Chlorophyll 'a' is primary pigment of photosynthetic process and important for plants growth and development. In the present investigation, Chlorophyll 'a'content in chickpea leaves increased up to 45 DAS and later decreased due to leaf senescence. At 45 DAS maximum Chlorophyll 'a'content was

found in plants sprayed with10 ppm Kinetin at 35DAS, which exhibited 40.4 per cent higher performance followed by spray of Ethrel at 25 DAS and Kinetin at 35 DAS and in single spray of Ethrel that showed an increase of 31.7 and 30.8 percent respectively compared to control.The above results sounds same with the findings of Bollivar[5] who reported the increase of chlorophyll in Maize seedlings treated with Kinetin.

Table 2.1: Effect of foliar sprays of Ethrel, Kinetin and Boron on Chlorophyll ' a' content (mg g^{-1}) of Chickpea leaves

Treatments	Chlorophyll ' a' content (mg g^{-1})			
	Days After Sowing			
	15	30	45	60
T_1: Ethrel @ 250 ppm at 25 DAS	0.64	0.91	1.36	0.95
T_2: Kinetin @ 10 ppm at 35 DAS	0.81	0.99	1.46	1.13
T_3: Boron @ 0.25% at 45 DAS	0.52	0.89	1.04	0.77
T_4: Ethrel @ 250 ppm at 25DAS + Kinetin @ 10 ppm at 35DAS	0.65	0.91	1.37	1.05
T_5: Kinetin @ 10 ppm at 35DAS + Boron @ 0.25% at 45DAS	0.60	0.90	1.15	0.82
T_6: Ethrel @ 250 ppm at 25DAS + Boron @ 0.25% at 45DAS	0.56	0.89	1.12	0.78
T_7: Ethrel @ 250 ppm at 25DAS + Kinetin @ 10 ppm at 35DAS + Boron @ 0.25% at 45DAS	0.62	0.90	1.20	0.88
T_8: Control	0.51	0.88	1.04	0.71
SEm +	**0.04**	**0.02**	**0.09**	**0.06**
CD (P= 0.05)	**0.13**	**0.07**	**0.27**	**0.19**
CV (%)	**12.04**	**4.19**	**12.65**	**12.52**

Chlorophyll 'b'

In chlorophyll 'b' contentchickpea plants showed significant differencesat all stages and it increased upto 45 DAS and then decreased (Table 2.2).

At 30 DAS, chlorophyll 'b' of chickpea plants with Ethrel spray varied from 0.28 to 0.50 mg g^{-1} with an average of 0.42 mg g^{-1} and it was higher than control (0.25 mg g^{-1}).At 45 DAS, higher chlorophyll 'b' content was observed with spray of 10 ppm Kinetin at 35 DAS (T_2 -0.74 mg g^{-1}). This was on par with the effect of250 ppm Ethrel at 25DAS + 10 ppm Kinetin at 35DAS (T_4 -0.70 mg g^{-1}) and 250 ppm Ethrel at 25DAS (T_1-0.61 mg g^{-1}). But in remaining treatments chlorophyll 'b' content was similar to control (T_8 -0.50 mg g^{-1}).

At 60 DAS, the chlorophyll 'b' content decreased and it ranged from 0.24 to 0.60 mg g^{-1}. Spray of 10 ppm Kinetin at 35 DAS (T_2), found superior to remaining treatments, which recorded the chlorophyll 'b' value of 0.60 mg g^{-1} and it was on par with the sprays of 250 ppm Ethrel at 25DAS + 10 ppm Kinetin at 35DAS (T_4 -0.54 mg g^{-1}). No significant differences were observed among the remaining treatments and were on par with control (T_8 -0.24 mg g^{-1}).

Chlorophyll 'b' is also an important pigment involved in photosynthetic activity of chloroplasts. It increased upto 45 DAS and later decreased. At 45 DAS maximum chlorophyll 'b'content was observed withT_2, T_4 and T_1, which exhibited an increase of 48,40 and 22 percent respectively compared to control. The above results sounds same with the findings of Reddy *et al*[30].

Table 2.2: Effect of foliar sprays of Ethrel, Kinetin and Boron on Chlorophyll ' b' content (mg g^{-1}) of Chickpea leaves

Treatments	Chlorophyll ' b' content (mg g^{-1})			
	Days After Sowing			
	15	30	45	60
T_1: Ethrel @ 250 ppm at 25 DAS	0.37	0.46	0.61	0.46
T_2: Kinetin @ 10 ppm at 35 DAS	0.22	0.52	0.74	0.60
T_3: Boron @ 0.25% at 45 DAS	0.27	0.27	0.52	0.26
T_4: Ethrel @ 250 ppm at 25DAS + Kinetin @ 10 ppm at 35DAS	0.25	0.50	0.70	0.54
T_5: Kinetin @ 10 ppm at 35DAS + Boron @ 0.25% at 45DAS	0.20	0.42	0.56	0.34
T_6: Ethrel @ 250 ppm at 25DAS + Boron @ 0.25% at 45DAS	0.17	0.28	0.53	0.30
T_7: Ethrel @ 250 ppm at 25DAS + Kinetin @ 10 ppm at 35DAS + Boron @ 0.25% at 45DAS	0.23	0.45	0.60	0.43
T_8: Control	0.23	0.25	0.50	0.24
SEm +	**0.02**	**0.03**	**0.04**	**0.02**
CD (P= 0.05)	**0.05**	**0.09**	**0.13**	**0.07**
CV (%)	**12.52**	**12.42**	**12.74**	**12.66**

Carotenoid content:

The variation in carotenoid content in leaf of chickpea plants influenced by Ethrel, Kinetin and Boron (Table 2.3) indicated that the differences among treatments were significant at 30 DAS and onwards.

At 30 DAS, carotenoid content in leaves with Ethrel spray varied from 0.70 to 0.84 mg g^{-1} with an average of 0.77 mg g^{-1}, and it was on par with control (0.64 mg g^{-1}). At 45 DAS, higher carotenoid content recorded with spray of 10 ppm Kinetin at 35 DAS (T_2 -1.03 mg g^{-1}), which exhibited 47.1 per cent higher performance compared to control. Followed by foliar spray of 250 ppm Ethrel at 25DAS + 10 ppm Kinetin at 35DAS (T_4 -0.94 mg g^{-1}) and 250 ppm Ethrel at 25DAS (T_1-0.90 mg g^{-1}), these showed an increase of 14.0 and 13.4 percent respectively compared to control (T_8 - 0.70 mg g^{-1}). The increase in carotenoid content from 30-45 DAS was 0.10 mg g^{-1} with Ethrel spray, 0.13 mg g^{-1} with Kinetin spray and 0.12 mg g^{-1} with Ethrel + Kinetin spray.

At 60 DAS, the carotenoid content decreased and it ranged from 0.51 to 0.80 mg g^{-1}. Kinetin spray @10ppm at 35 DAS (T_2 - 0.80 mg g^{-1}) recorded higher value followed by T_4 (0.73 mg g^{-1}) and T_1 (0.70 mg g^{-1}). The effect of other treatments (T_7, T_5, T_6 and T_3) was statistically similar to control.

Table 2.3: Effect of foliar sprays of Ethrel, Kinetin and Boron on Carotenoid content (mg g^{-1}) of Chickpea leaves

Treatments	Carotenoid content (mg g^{-1})			
	Days After Sowing			
	15	30	45	60
T_1: Ethrel @ 250 ppm at 25 DAS	0.56	0.80	0.90	0.70
T_2: Kinetin @ 10 ppm at 35 DAS	0.65	0.89	1.03	0.80
T_3: Boron @ 0.25% at 45 DAS	0.42	0.64	0.72	0.53
T_4: Ethrel @ 250 ppm at 25DAS + Kinetin @ 10 ppm at 35DAS	0.61	0.84	0.94	0.73
T_5: Kinetin @ 10 ppm at 35DAS + Boron @ 0.25% at 45DAS	0.52	0.72	0.83	0.55
T_6: Ethrel @ 250 ppm at 25DAS + Boron @ 0.25% at 45DAS	0.49	0.70	0.80	0.53
T_7: Ethrel @ 250 ppm at 25DAS + Kinetin @ 10 ppm at 35DAS + Boron @ 0.25% at 45DAS	0.53	0.74	0.88	0.63
T_8: Control	0.40	0.64	0.70	0.51
SEm +	**0.04**	**0.05**	**0.06**	**0.05**
CD (P= 0.05)	**0.11**	**0.16**	**0.18**	**0.14**
CV (%)	**12.13**	**12.39**	**12.05**	**12.84**

Total chlorophyll

The total chlorophyll in chickpea plants increased upto 45 DAS and then declined (Table 2.4).At 30 DAS, total chlorophyll content with Ethrel spray varied from 1.24 to 1.68 mg g^{-1} with an average of 1.47 mg g^{-1} and it was found on par with control (1.22 mg g^{-1}). However, the increase in total chlorophyll content from 15-30 DAS with Ethrel spray at 25 DAS was more (0.62 to 0.97 mg g^{-1} with an average of 0.79 mg g^{-1}) compared to control (0.68 mg g^{-1}).

At 45 DAS, higher total chlorophyll content recorded with spray of 10 ppm Kinetin at 35 DAS (T_2 -1.82 mg g^{-1}). In remaining treatments it varied from 1.59 to 1.79 mg g^{-1} and the least was observed in control (T_8 -1.57 mg g^{-1}).

At 60 DAS, it decreased and ranged from 0.78 to 1.67 mg g^{-1}. Among the treatments 10 ppm Kinetin at 35 DAS (T_2 - 1.67 mg g^{-1}) recorded higher total chlorophyll content. The effect of other treatments (T_4, T_1, T_7 ,T_5, T_6 and T_3) was numerically superior to control (T_8 -0.78 mg g^{-1}) but differences among them were on par.

The spray of Kinetin exhibited high performance in increasing the total chlorophyll content 15.9 per cent over control followed by combination of Ethrel at 25 DAS + Kinetin at 35 DAS and Ethrel at 25 DAS, which exhibited 14.0 and 13.4 per cent increase.

Spray of Kinetin exhibited high performance in increasing the total chlorophyll content. The above results were concurrent with the findings of Bollivar[5] in maize. Thakur *et al.*[37], reported the increase in chlorophyll content in leaves of *Brassica napus* with Ethrel spray. Grewal *et al.*[17], reported that the exogenous application of Ethrel in mustard seedlings enhanced chlorophyll synthesis considerably.

Table 2.4: Effect of foliar sprays of Ethrel, Kinetin and Boron on Total Chlorophyll content (mg g^{-1}) of Chickpea leaves

Treatments	Total Chlorophyll content (mg g^{-1})			
	Days After Sowing			
	15	**30**	**45**	**60**
T_1: Ethrel @ 250 ppm at 25 DAS	0.70	1.53	1.78	0.96
T_2: Kinetin @ 10 ppm at 35 DAS	0.83	1.76	1.82	1.67
T_3: Boron @ 0.25% at 45 DAS	0.54	1.22	1.59	0.88
T_4: Ethrel @ 250 ppm at 25DAS + Kinetin @ 10 ppm at 35DAS	0.71	1.68	1.79	0.98
T_5: Kinetin @ 10 ppm at 35DAS + Boron @ 0.25% at 45DAS	0.65	1.31	1.72	0.92
T_6: Ethrel @ 250 ppm at 25DAS + Boron @ 0.25% at 45DAS	0.62	1.24	1.65	0.90
T_7: Ethrel @ 250 ppm at 25DAS + Kinetin @ 10 ppm at 35DAS + Boron @ 0.25% at 45DAS	0.70	1.42	1.76	0.94
T_8: Control	0.54	1.22	1.57	0.78
SEm + –	**0.05**	**0.11**	**0.05**	**0.07**
CD (P= 0.05)	**0.14**	**0.32**	**0.16**	**0.22**
CV (%)	**12.38**	**12.94**	**5.43**	**12.32**

Nitrate reductase activity (µM$NO_2^-$$g^{-1}$ hr^{-1})

Significant differences were observed at all stages of plant growth in nitrate reductase activity (NRA) of chickpea leaves with the influence of Ethrel, Kinetin and Boron (Table 3).

At 30 DAS, higher NRA recorded with spray of Ethrel varied from 0.2 to 0.24with an average of 0.22 µM $NO_2^-g^{-1}$ hr^{-1} and found higher than control (0.17 µM $NO_2^-g^{-1}$ hr^{-1}).

At 45 DAS, significantly higher value recorded with spray of 10 ppm Kinetin at 35 DAS (T_2 -3.65 µM $NO_2^-g^{-1}$ hr^{-1}). In remaining treatments (T_4, T_7 and T_5) it varied from 1.64 to 2.79 µM $NO_2^-g^{-1}$ hr^{-1}, and superior to control

(T_8 -1.02 µM $NO_2^-g^{-1}$ hr^{-1}).The effect of other treatments was on par with control. The increase in NRA of chickpea plants from 30-45 DAS was 1.04 µM $NO_2^-g^{-1}hr^{-1}$ with Ethrel spray, 2.92 µM $NO_2^-g^{-1}$ hr^{-1} with Kinetin spray and 1.47 µM $NO_2^-g^{-1}$ hr^{-1} with Ethrel + Kinetin spray.

At 60 DAS, all treatments differed significantly with each other. The NRA of chickpea plants increased and ranged from 1.96 to 3.37µM $NO_2^-g^{-1}$ hr^{-1}. Spray of 10 ppm Kinetin at 35 DAS (T_2 - 3.37 µM $NO_2^-g^{-1}$ hr^{-1}) was on par with the spray of 10 ppm Kinetin at 35DAS + 0.25% Boron at 45DAS (T_5 -2.88 µM $NO_2^-g^{-1}$ hr^{-1}), and superior to remaining treatments. No significant differences were observed among the remaining treatments and were on par with and with control (T_8 -1.96).

Increase in NRA in Kinetin sprayed plants might be due to enhancement of nitrogen uptake by plant. It might also be due to the positive effect of cytokinin in nodulation[15,25,38].

Table 3: Effect of foliar sprays of Ethrel, Kinetin and Boron on Nitrate Reductase Activity (µMNO_2^- g^{-1} hr^{-1}) of Chickpea leaves

Treatments	Nitrate Reductase Activity			
	Days After Sowing			
	15	30	45	60
T_1: Ethrel @ 250 ppm at 25 DAS	0.31	0.34	3.65	3.37
T_2: Kinetin @ 10 ppm at 35 DAS	0.22	0.24	1.61	2.50
T_3: Boron @ 0.25% at 45 DAS	0.23	0.24	1.64	2.55
T_4: Ethrel @ 250 ppm at 25DAS + Kinetin @ 10 ppm at 35DAS	0.28	0.27	2.79	2.88
T_5: Kinetin @ 10 ppm at 35DAS + Boron @ 0.25% at 45DAS	0.22	0.21	1.27	2.47
T_6: Ethrel @ 250 ppm at 25DAS + Boron @ 0.25% at 45DAS	0.23	0.24	1.78	2.63
T_7: Ethrel @ 250 ppm at 25DAS + Kinetin @ 10 ppm at 35DAS + Boron @ 0.25% at 45DAS	0.14	0.17	1.02	1.96
T_8: Control	0.02	0.02	0.14	0.19
SEm + **–**	**0.05**	**0.05**	**0.42**	**0.57**
CD (P= 0.05)	**11.50**	**11.92**	**12.70**	**12.55**
CV (%)	**0.31**	**0.34**	**3.65**	**3.37**

4.3.6. Total soluble protein (mg g^{-1}) of seeds

Total soluble protein content in seeds of chickpea plants treated with Ethrel, Kinetin and Boron indicated that, there was significant difference among the treatments (Table 4).

High protein content of seeds was shown by the spray of 250 ppm Ethrel at 25DAS + 10 ppm Kinetin at 35DAS + 0.25% Boron at 45DAS (T_7 -128.2 mg g^{-1}), which was on par with 10 ppm Kinetin at 35 DAS (T_2 -121.1 mg g^{-1}), 10 ppm Kinetin at 35DAS + 0.25% Boron at 45DAS (T5-120.7 mg g^{-1}) and with foliar spray of250 ppm Ethrel at 25DAS + 10 ppm Kinetin at 35DAS (T_4 -119.5 mg g^{-1}). Low protein content of seed was recorded in control (T_8-109.6 mg g^{-1}). The effect of other treatments (T_4, T_3, T_6 and T_1) on total soluble protein content was on par with control and also the differences among them were nonsignificant.

Maximum protein content recorded in spray of 250 ppm Ethrel at 25 DAS +10 ppm Kinetin at 35DAS + 0.25% Boron at 35 DAS was 17 per cent higher compared to control. Gadallah and Sayed[12] reported higher soluble proteins and total free amino acids in Kinetin treated sorghum plants. Gupta *et al.*[18], studied the effect of cytokinin application on modulation of morpho physiological responses of wheat genotypes and found an increase in protein content.

Chanekar*et al.*[6], reported that seed protein content was significantly increased by 14% in pigeon pea with the application of

Ethrel @ 50 ppm. Rizwan Zahoor*et al*.[31], observed the increase in protein content in sunflower with spray of Boron (2 kg ha^{-1}) at ray floret stage and button stage.

Total Starch content (mg g^{-1}) of seeds

Application of Ethrel, Kinetin and Boron contributed significant difference in total starch content in seeds of chickpea plants (Table 4) over control and that varied from 178.3 to 386.7 mg g^{-1}. Foliar spray of 0.25% Boron at 45 DAS (T_3- 386.7 mg g^{-1}) recorded the highest starch content which was on par with T_5 (375.4 mg g^{-1}) and T_7 (369.3 mg g^{-1}). The lowest was observed in control and it was on par with the effect of Ethrel spray at 25 DAS. The influence of the treatments T_2, T_6 and T_4 was superior to control.

In chickpea seeds, highest value of starch content recorded with spray of 0.25% Boron at 45 DAS followed by Kinetin + Boron and Ethrel + Kinetin + Boron. This might be due to the active role of Boron in translocation of metabolites from source-sink as reported by Chatterjee[7] and Pratima Sinha *et al.*[29].

4.3.8. Fe and Zn content of seeds

Effect of Ethrel, Kinetin and Boron sprays on Fe and Zn content of chickpea seeds exhibited significant differences among the treatments (Table 4).

Iron content in seed varied from 45.4 to 62.2 µg g^{-1}. Foliar spray of 0.25% Boron at 45 DAS (T_3- 62.2 µg g^{-1}) resulted in significant increase in Fe content of seed compared to control and other treatments except 10 ppm Kinetin at 35DAS + 0.25% Boron at 45DAS (T_5-53.0 µg g^{-1}) with which it was on par. The lowest was recorded with control (T_8 – 45.4 µg g^{-1}), and it was on par with the remaining treatmentsT_7, T_2, T_6, T_4 and T_1 (45.5 to 51.7 µg g^{-1}).

Table 4: Effect of foliar sprays of Ethrel, Kinetin and Boron on Total Protein (mg g^{-1}), Starch (mg g^{-1}), Fe (µg/g) and Zn (µg/g) content of Chickpea seeds

Treatments	Total Protein (mg g^{-1})	Starch (mg g^{-1})	Fe (µg/g)	Zn (µg/g)
T_1: Ethrel @ 250 ppm at 25 DAS	111.36	225.56	45.52	30.17
T_2: Kinetin @ 10 ppm at 35 DAS	121.10	312.49	49.67	30.70
T_3: Boron @ 0.25% at 45 DAS	115.27	386.73	62.25	64.90
T_4: Ethrel @ 250 ppm at 25DAS + Kinetin @ 10 ppm at 35DAS	119.52	252.07	46.62	30.47
T_5: Kinetin @ 10 ppm at 35DAS + Boron @ 0.25% at 45DAS	120.68	375.42	53.03	58.63
T_6: Ethrel @ 250 ppm at 25DAS + Boron @ 0.25% at 45DAS	114.90	278.33	49.04	28.53
T_7: Ethrel @ 250 ppm at 25DAS + Kinetin @ 10 ppm at 35DAS + Boron @ 0.25% at 45DAS	128.21	369.27	51.69	52.00
T_8: Control	109.61	178.25	45.37	22.93
SEm + —	**3.59**	**21.28**	**3.26**	**2.65**
CD (P= 0.05)	**10.87**	**64.54**	**9.89**	**8.04**
CV (%)	**5.28**	**12.40**	**11.20**	**11.55**

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