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FERTI-FORTIFICATION: AN EASY APPROACH FOR NUTRITIONAL ENRICHMENT OF CHICKPEA

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ABSTRACT

A field experiment was conducted to study the effect of variable moisture regimes and diverse combinations of foliar fertilization on growth, yield attributes, yield, nutrient partitioning and nutritional enrichment of chickpea. Three supplemental irrigation (No irrigation, one irrigation, two irrigation) and eight foliar fertilization (water spray, urea, Fe, Zn, Fe+urea, Zn+urea, Fe+Zn and Fe+Zn+urea) treatments were tested in sandy loam soil (*Typic Ustochrept*). Significantly taller plants, higher pods, grains, 1,000 grain weight were observed due to application of one irrigation over no irrigation. Likewise, one irrigation also had marked effect on grain yield (17.81 to 19.84 q/ha) over no irrigation (13.68 to 15.73 q/ha). Further, foliar fertilization or ferti-fortification with combination of urea+zinc+iron had highest influence on nutrient concentration (N, protein, zinc and iron) enrichment of chickpea grain. Thus, the inference of the findings is that applying one irrigation and combined ferti-fortification (urea+iron+zinc) could be the potential strategy for attaining higher yield and nutritional security of the vegetarian population in India.

KEY WORDS
Chickpea
Ferti-fortification
Foliar Spray
Micronutrient
Urea

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INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most important food legume or 'pulse crop' in the world with production of 13.11 million tons from an acreage of 13.57 million hectares with the productivity of 9.6 q/ha (FAOSTAT, 2013). India is world's leading producer of chickpea accounting for nearly 65.78 and 67.35 percent of the total area (8.52 million hectares) and production (8.83 million tons), respectively (DAC, 2015). Unlike plants, humans also require essential micronutrients and protein for normal physiological functions of the body and general health. Due to low concentration of micronutrients and protein in the staple food, billions of population is lacking sufficient daily intake of micronutrient and protein in their diet (Thavarajah and Thavarajah, 2012; Singh *et al.,* 2015). The traditional strategies of delivering mineral micronutrients to the human being relied on food fortification, dietary diversification and mineral supplementation (White and Broadly, 2005). These efforts have had limited success due to one or another reason. An alternative solution to micronutrient and protein malnutrition is ferti-fortification. The strategies of 'biofortification' through traditional breeding or transgenics are equally good, but time consuming. Yields of chickpea are low and variable in many parts of the country and it is important to find the ways to enhance yield. Low level of chickpea yield may be attributed to its major cultivation under rainfed condition or non-irrigated soils, imbalance use of fertilizers without micronutrients. Non-irrigated soils are low in native fertility. Although chickpea is a hardy plant, a poor supply of water or moisture along with nutrient deficiencies in soil are leading stresses aggravates yield loss and low nutrient concentration in the grain. Micronutrients that contribute substantially to higher nitrogen fixation by legume-rhizobium symbioses which also have marked influence on uptake of major and secondary nutrients (Roy *et al.,* 2013). At later growth stage roots of chickpea generally fails to absorb nitrogen from the soil. Due to lower nitrogen supply the translocation of N to leaves and grain is also affected. Urea have the potential to increase the concentration of storage N compounds like amino acids and proteins thereby urea spray directly influence N metabolism and amino acid synthesis under normal conditions. Due to high leaf penetration rate, rapid absorption, faster hydrolysis in cytosol and low cost, urea is most preferred as foliar N-fertilizers (Witte *et al.*, 2002). Moreover, direct application of N-fertilizers to leaves, especially urea, can be a potential alternative to conventional soil fertilization when the application of N-fertilizer due to any cause is not effective or the N_2-fixation has been suppressed (Aliloo *et al.*, 2012). Therefore, foliar nutrition through urea may be a practical solution to enhance N or protein concentration in the grain.

Further, Zn deficiency is widespread in chickpea growing region of the world and is most prevalent among the micronutrients. Zinc enhances water use and water use efficiency (Khan *et al.,* 2004), nodulation and nitrogen fixation. Furthermore, zinc is important for over all plant growth and development (Reddy *et al.,* 2014). High soil pH, low organic matter content, more sand leads to lower yields due to poor utilization of zinc. Zinc is involved in a wide variety of metabolic processes, including carbohydrate, lipid, protein and nucleic acid synthesis and degradation. As well it can substantially improve seed germination and seedling vigour (Auld, 2001).

Iron is one the most essential micro-nutrient in plants and is a component of some antioxidant enzymes which are involved in the protection of chloroplasts from free radicals (Janmohammadi *et al.,* 2012). Also, iron could be considered as a constituent of the heme group that is a precursor of chlorophyll. The uptake of Fe is also affected by varying soil factors. Foliar spray of Fe could be one of the most efficient techniques in preventing and remedying iron deficiency in plants including chickpea.

Although few researchers have separately studied various aspects of foliar fertilization and supplemental irrigation, the interaction of micronutrients and supplemental irrigation is less understood. Therefore, the objective of the research was to investigate the influence of foliar spray of micronutrient (Fe and Zn) and urea separately and in combination along with irrigation on productivity and nutrient enrichment of chickpea.

MATERIALS AND METHODS

A field experiment was conducted during two consecutive *rabi* seasons of 2012-13 and 2013-14 at New Research Farm, ICAR-Indian Institute of Pulses Research, Kanpur, Uttar Pradesh. The objective of the research was to study the response of supplemental irrigation and foliar nutrition on growth, yield attributes, yield and nutritional enrichment of chickpea. Geographically, the current experimental site falls under sub-tropical zone in Indo-Gangetic Plains with alluvial calcareous soils lies on 26.46ºN latitude and 80.35ºE longitude at an altitude of 126 meters above mean sea level. The climate is tropical sub-humid with an annual rainfall of 722 mm and mean annual maximum and minimum temperature of 33.0 and 20.0ºC respectively. The experimental site was well drained and soil was sandy loam (*Typic Ustochrept*) and non-saline (EC 0.63 dS/m) with pH 8.31 (1:2.5 soil:water) bulk density 1.41 g/cc and contained 0.23% organic carbon, 203.8 kg/ha available N, 11.5 kg/ha 0.5 M $NaHCO_3$ extractable P, 151.2 kg/ha available K, 9.6 kg/ha available S, 0.63 mg/kg DTPA extractable Zn and 1.63 mg/kg Fe. The experiment was conducted in a split plot design and replicated thrice. Treatments consisted of three supplemental irrigation levels (No irrigation, one irrigation and two irrigation) in the main plot and eight foliar fertilization (water spray, urea, Fe, Zn, Fe+urea, Zn+urea, Fe+Zn and Fe+Zn+urea) in sub-plots. Chickpea 'DCP 92-3' was sown second week of November spaced at 30×10cm apart using 60 kg seed/ha during both the years of experimentation. The crop was supplied recommended dose of nitrogen (20 kg N/ha), phosphorus (50 kg P_2O_5/ha), potassium (20 kg K_2O/ha) and sulphur (20 kg S/ha) through urea (subtracted the amount of N supplied from DAP), di-ammonium phosphate, muriate of potash and elemental sulphur, respectively as basal. Treatments were applied as per standard methods. Supplemental irrigation (5cm) was applied to the respective plots as per the treatments. Foliar application of Urea, Zn and Fe was done through 2% Urea (46% N), 0.5% $ZnSO_4.7H_2O$ (23% Zn) and 0.3% $FeSO_4.H_2O$ (20% Fe) using 400 litre of water/ha as per treatments. All solution contained 0.01% (v/v) Tween as a surfactant. The foliar treatments were applied at flowering initiation of chickpea in the afternoon (16:00 to 18:00 hours) under sunshine and windless condition. At all the stages most of the solution was captured by the chickpea plants. Gap filling and thinning were done wherever necessary to maintain the plant population. The weeds were controlled by pre-emergence spray of Pendimethalin @ 1.0 litre/ha and supplemented with one hand weeding at 40 days after sowing. Observations on growth characters, yield attributes and yield were recorded using standard procedure. At harvest, samples of grain and stover were drawn from each plot of the experiment for the chemical analysis of N, Zn and Fe concentration. Nitrogen was analysed through Kjeldahl method and the analysd N was multiplied by 6.25 to obtain the protein. Zinc and iron in grain and plant samples were analyzed content on a di-acid ($HClO_4$ + HNO_3 in 3:10 ratio) digest on an Atomic Absorption Spectrophotometer (Prasad *et al.,* 2006). All the data were statistically analyzed using F-test as per the standard procedure. The data recorded on different parameters were pooled, as the differences between the years were not significant.

RESULTS AND DISCUSSION

Growth and yield attributes

Application of supplemental irrigation significantly influenced growth and yield attributes (Table 1). Plant height, number of branches/plant, number of pods/plant, grains/pod and 1,000 grain weights were significantly higher when chickpea was supplemented with one irrigation over no irrigation. However, further increase in number of irrigation (two irrigation) markedly enhanced chickpea height (75.4 cm) although had not improved yield attributes over one irrigation. It could be attributed to the fact that liberal water supply promoted more vegetative growth at the expense of pod formation, as evidenced by enhanced plant height. However, two irrigations being on par with one irrigation markedly enhanced plant height, branches/plant, pods/plant, grains/pod and 1,000 grain weight over no irrigation. This indicates that liberal supply of irrigation water had no scope for improvement in yield attributes (Ahlawat *et al.*, 2005).

Foliar fertilization of chickpea either with urea, zinc, iron or combination thereof markedly improved plant height, branches, pods/plant, grains/pod and 1,000 grain weight (Table 1). Foliar application of urea alone significantly enhanced chickpea height over iron, zinc and Zn+Fe spray. Even combined spray of urea as urea+Zn, urea+Fe and urea+Zn+Fe had marked improvement in plant height over sole spray of zinc or iron and zinc+iron spray. The significant increase in chickpea height due to urea spray might be due to more availability of nitrogen and water to the plants through spray (Verma *et al.*, 2009). Among foliar fertilization practices, combined spray of urea+Zn+Fe recorded greatest number of branches, pods/plant, grains/pod and 1,000 grain weight over Fe+Zn, Zn+urea, Fe+urea, Zn, Fe and urea spray. This could be attributed to the fact that chickpea plant is capable of absorbing soluble compounds through leaves, by which nutrients are delivered through foliar fertilization. Applying

Table 1: Growth and yield attributes of chickpea as influenced by foliar fertilization and variable moisture regime

Treatment	Plant height at harvest (cm)	Branches/ plant	Pods /plant	Grains/ pod	1,000 grain weight (g)
Irrigation					
No Irrigation	57.7	3.2	61.1	1.92	165.30
One Irrigation	66.7	3.7	78.5	2.36	195.50
Two irrigation	75.4	3.8	79.4	2.38	196.60
CD ($P=0.05$)	4.2	0.21	4.6	0.18	21.3
Foliar Fertilization					
Water Spray	58.7	3.2	61.2	1.91	166.20
Urea	67.1	3.6	74.8	2.26	183.48
Fe	61.2	3.3	67.1	2.12	171.98
Zn	61.4	3.3	67.4	2.16	173.08
Fe + Urea	71.5	3.7	79.8	2.34	198.28
Zn + Urea	72.1	3.7	80.2	2.37	200.38
Fe + Zn	65.4	3.5	71.2	2.22	188.18
Fe + Zn + Urea	75.6	4.1	83.2	2.41	205.08
CD ($P=0.05$)	3.3	0.13	2.8	0.11	16.4

Table 2: Yield of chickpea as influenced by foliar fertilization and variable moisture regime

Treatment	Biological yield (q/ha)	Stover yield (q/ha)	Grain yield (q/ha)		Harvest index
			2012-13	2013-14	
Irrigation					
No Irrigation	39.06	25.38	15.73	13.68	0.35
One Irrigation	44.86	28.21	18.51	16.65	0.37
Two irrigation	45.51	28.78	18.60	16.73	0.37
CD ($P=0.05$)	3.8	2.6	0.62	1.67	NS
Foliar Fertilization					
Water Spray	39.24	25.11	15.07	14.13	0.36
Urea	41.80	26.52	17.29	15.28	0.37
Fe	40.53	25.61	16.14	14.92	0.37
Zn	41.04	26.06	16.44	14.98	0.37
Fe + Urea	42.87	27.04	18.91	15.83	0.37
Zn + Urea	45.02	28.34	19.37	16.68	0.37
Fe + Zn	43.09	27.33	17.83	15.76	0.37
Fe + Zn + Urea	47.19	29.38	19.84	17.81	0.38
CD ($P=0.05$)	2.2	1.7	0.41	0.46	NS

nutrients through foliar fertilization implies that nutrients are being absorbed and exported from leaves (point of application) to growing tissues (point of consumption). Application source, either urea, zinc or iron foliarly might helped in better photosynthesis and photosynthate partitioning to yield attributing characters which resulted in higher sink size (Ferrandon and Chamel, 1988; Ghasemi-Fasaei *et al.,* 2005; Shivay *et al.,* 2013).

Yield

Data pertaining to biological yield, stover yield, grain yield and harvest index are presented in Table 2. Applying one irrigation gave significantly more yield (Biological, stover and grain yield) over no irrigation. However, a further increase in number of irrigation had no significant effect on stover and grain yield during both the years of experimentation. The increase in seed yield with one irrigation over no irrigation being 17.6% in first and 21.7% in second year. This increase in seed yield was attributes to increased yield attributes (Srivastava and Srivastava, 1994; Ahlawat *et al.,* 2005). Contrary to this harvest index was not influence by irrigation levels.

Among foliar treatments (Table 2), combined spray of urea+Zn+Fe recorded maximum yield (stover and grain). The foliar spray was done at the crop stage of pre-flowering. In general, grain yield was more in first year as compared to second year. Foliar spray of urea enhanced grain yield from 8.13-14.7 per cent over water spray. Likewise, zinc and iron spray enhanced grain yield to the tune of 5.5-7.1 and 6.0-9.0 per cent, respectively. Furthermore, foliar fertilization with urea+Fe+Zn enhanced grain yield by 26.0-31.0 per cent. Akay (2011) and Das *et al.* (2012) also reported yield advantages due to Zinc and iron spray in chickpea. It has was noticed that nodule degeneration had started after 60 days after sowing of crop stage (data not presented), thereby lowering the nitrogen availability in the leaves of chickpea. This deficit arises out of nodule degeneration and declining nitrogen availability could be one of the reason for enhanced grain yield of chickpea (Venkatesh and Basu, 2011; Gupta *et al.,* 2011). Combined spray of urea+Zn+Fe enhanced the grain yield to the highest level (1781-1984 kg/ha) over rest of the spray treatments. Nitrogen, zinc and iron have imperative role in normal growth and development of plant. Zinc, being an important component of number of enzymes plays vital role in metabolism of nitrogen, protein synthesis, precursor of auxin etc. Iron on the other hand also play important role in

Table 3: Effect of foliar fertilization and variable moisture regime on nutrient concentration (mg/kg) of different plant parts of chickpea at maturity

Treatment	Leaf			Stem			Root		
	N	Zn	Fe	N	Zn	Fe	N	Zn	Fe
Irrigation									
No irrigation	3.23	37.14	52.40	1.08	12.47	17.59	1.94	22.29	31.44
One irrigation	4.11	45.74	59.33	1.38	15.35	19.92	2.47	27.44	35.60
Two irrigation	4.26	49.20	59.74	1.43	16.52	20.05	2.55	29.52	35.84
CD ($P=0.05$)	0.64	3.28	5.78	0.21	1.10	1.94	0.35	1.97	3.47
Foliar fertilization									
Water spray	3.50	37.97	50.65	1.18	12.75	17.00	2.10	22.78	30.39
Urea	4.11	40.80	52.09	1.38	13.70	17.49	2.46	24.48	31.26
Fe	3.52	39.51	61.75	1.18	13.26	20.73	2.11	23.70	37.05
Zn	3.53	45.47	52.04	1.19	15.27	17.47	2.12	27.28	31.22
Fe+Urea	4.15	40.89	61.78	1.39	13.73	20.74	2.49	24.54	37.07
Zn+Urea	4.17	49.34	52.28	1.40	16.56	17.55	2.50	29.60	31.37
Fe+Zn	3.55	45.51	61.94	1.19	15.28	20.79	2.13	27.31	37.16
Fe+Zn+Urea	4.42	52.25	64.88	1.48	17.54	21.78	2.65	31.35	38.93
CD ($P=0.05$)	0.41	2.46	2.95	0.16	0.83	0.99	0.22	1.48	1.77

Table 4: Effect of foliar fertilization and moisture regime on grain quality parameters of chickpea

Treatment	Zn (mg/kg)	Fe (mg/kg)	Protein (%)
Irrigation			
No irrigation	26.53	37.43	14.42
One irrigation	32.67	42.38	18.36
Two irrigation	35.14	42.67	19.01
CD ($P=0.05$)	2.34	4.13	0.68
Foliar fertilization			
Water spray	27.12	36.18	15.64
Urea	29.14	37.21	18.34
Fe	28.22	44.11	15.72
Zn	32.48	37.17	15.76
Fe+Urea	29.21	44.13	18.51
Zn+Urea	35.24	37.34	18.62
Fe+Zn	32.51	44.24	15.83
Fe+Zn+Urea	37.32	46.34	19.73
CD ($P=0.05$)	1.76	2.11	0.31

chlorophyll synthesis, besides helping in absorption of other nutrients. It may be the reason for enhanced yield attributing characters and yield, as combined spray of N, Fe and Zn have cohesion and synergistic effect (Marschner, 1995; Janmohammadi *et al.*, 2012).

Nutrient partitioning in plant

Application of irrigation enhanced N, Fe and Zn concentration in all the plant parts of chickpea (Table 3). Water more than twice failed to influence N, zinc and iron concentration in chickpea leaves, stem as well as roots over one watering. This might be due to attaining sufficiency level of nutrient absorption. The highest concentration of N, iron and zinc was observed in leaf, followed by root and least in stem. As nutrient concentration in plant parts is more effected by foliar feeding/ foliar spray rather than more watering (Bohra *et al.*, 2014; Khan *et al.*, 2003; Pingoliya *et al.*, 2014). The concentration of N, Zn and Fe was found more in leaves followed by roots and least in stem. This indicated that there is still scope of nutrient translocation form leaves (source) to grain (sink). Further studies are required to reveal the facts.

Foliar fertilization with urea, zinc or iron significantly improved the concentration in stem, leave and root of the chickpea plant (Table 3). Urea spray enhanced N concentration in leaves from 3.5% in control to 4.11 % in urea sprayed plot. Likewise, N concentration in stem and root was also improved due to urea spray. Combined spray of urea with either zinc or iron also had markedly enhanced N concentration in stem, leaves and root of the chickpea. The zinc concentration in leaves, stem and root at maturity increased significantly with zinc supply over no zinc supply. The extent of zinc concentration was more in all the plant parts when zinc was sprayed either with urea or iron. Highest zinc concentration was noticed due to combined spray of zinc +urea+ iron in chickpea leaves (52.25 mg/kg), stem (17.54 mg/kg) and roots (31.35 mg/kg). The reason for higher concentration in leaves may be due to the fact that at adequate nutrient supply, the leaves of the chickpea were a major reserve of zinc at maturity. As the site of application was foliage. These observations are consistent with the results of Kaya *et al.*, 2009; Khan *et al.*, 2000; Khan *et al.*, 2003. Further, foliar application of iron markedly enhanced iron concentration in chickpea leaves, stem and root over no spray. The order of iron concentration in chickpea plant parts were in the order: leaves (64.88 mg/ kg) > roots (38.93 mg/kg) > stem (21.78 mg/kg). The variation in total iron distribution within plant parts indicates scope of translocation of iron from leafs to grains. However, the combined spray of iron with zinc and urea had highest effect on iron concentration in plant parts over sole or no spray. As zinc, iron and nitrogen have imperative role in enhanced growth and thereby nutrient concentration in the plant parts (Janmohammadi *et al.*, 2012; Mahmoudi, *et al.*, 2005; Singh *et al.*, 2015).

Nutritional enrichment of chickpea grain

Application of irrigation to chickpea significantly improved zinc, iron and protein density in grain (Table 4). Watering twice to chickpea being on par with once that significantly enhanced zinc (35.14 mg/kg), iron (42.67 mg/kg) and protein (19.01 %) density in grain. The per cent increase in zinc, iron and protein content due to one irrigation over no irrigation was recorded to the tune of 23.1, 13.2 and 27.3, respectively. This indicates that well watered condition resulted in more translocation of N, Zn and Fe. Similar observations were also reported by Saxena *et al.* (1990) and Khan *et al.* (2003).

Further, foliar spray with urea or zinc or iron significantly enhanced concentration of protein, zinc and iron, respectively over no spray (Table 4). Foliar spray with urea alone improved protein content by 17.2 per cent over water spray. Likewise, zinc and iron spray enhanced zinc and iron concentration in chickpea grain by 19.7 and 21.9 per cent, respectively. Under sufficient moisture condition, the translocation of assimilated nitrogen in root-rhizobia symbiosis is enhanced; it might be reason for enhanced protein content in chickpea grain due to urea spray. Venkatesh and Basu (2011) and Verma *et al.* (2009) also reported the advantages of foliar urea spray in chickpea over water or no spray. Applying zinc foliarly significantly increased Zn concentration in grain when applied either alone or in combination with iron or urea or iron+urea+zinc. Without Zn spray, Zn concentration in grain recorded only 27.12 mg/kg. When zinc was sprayed the Zn concentration improved (32.48 mg/kg). When zinc+iron was sprayed together the zinc concentration observed was 32.51 mg/kg (Not much improvement). However, combined spray of zinc+iron+urea had marked jump (highest) in zinc concentration (37.32 mg/kg). It is attributed to the fact that nitrogen application may enhance zinc concentration in grain (Rengel *et al.,* 1999; Kumar *et al.,* 2011). Likewise, foliar spray of iron also had marked influence on iron concentration improvement in chickpea grain. Highest concentration of iron was observed when it was sprayed in combination with urea and zinc (46.34 mg/kg) followed by spray of zinc+iron (44.24 mg/kg), iron+urea (44.13 mg/kg), iron alone (44.11 mg/kg) and no iron spray (36.18 mg/kg). This indicates that combination of iron spray with either urea or zinc had no significant effect on iron concentration improvement in chickpea grain. Akay (2011) and Das *et al.* (2012) also reported advantages of zinc and iron spray. Although, combined spray of iron+zinc+urea significantly enhanced iron concentration in grain over preceding levels. This may be ascribed to the fact that mobilization of iron from leaves to grain is low compared with zinc. As iron has intermediate mobility within the phloem though zinc has considerably higher mobility within the phloem (Rengel *et al.,* 1999). These findings corroborate with that of Valenciano *et al.* (2011), Gupta and Sahu (2012).

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