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CONTENTS

Effect of Stabilized Magnetite Nano Fertilizer on growth, yield and nutrient contents of broccoli (*Brassica oleracea var. italica* L.) cv. F1 HYB NS-50

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ABSTRACT: A field experiment was carried out during *rabi* season 2020 to determine the effect of Magnetite Nano-Fertilizer (Fe_3O_4 NPs) stabilized with EDTA on growth, yield and nutrient content of broccoli (*Brassica oleracea L. var. Italica*). The Fe_3O_4 NPs were prepared and particle size analysis revealed that the particle size was of 40 nm. Experiment with spraying of three iron nutrient sources with four different concentrations (Fe EDTA, 300 ppm, $FeSO_4.7H_2O$, 195 ppm and EDTA stabilized Fe_3O_4 NPs, 6 ppm and 3 ppm) were conducted on Randomized Block Design (RBD) with three replications of crop. The results revealed that application of 3 ppm solution of stabilized Fe_3O_4 NPs to broccoli significantly increased the plant height, stalk length, total head yield per hectare and biological yield per hectare as compared to control treatment. The result indicated the significant influence of applying Fe_3O_4 NPs, 3 ppm followed by $FeSO_4.7H_2O$, 195 ppm. These micronutrients helped inincreasing plant height, stalk length, head weight per plant, head yield quintal per hectare and nutrient content in head with respect to control. Thus, stabilized Fe_3O_4 NPs, at 3 ppm foliar application seems to be a promising approach to maximize broccoli growth and head yield production.

Key words: Broccoli, EDTA stabilized, Fe_3O_4 NPs, Nano fertilizers, Yield increase

Growth of human population, urbanization and industrialization are various elements that have resulted in the decreasing of available agricultural land as well as the global agricultural productivity of crops. Hence, there is an immediate requirement for the innovative technologies to improve crop production. Development of novel fertilizers that can substitute the traditional methods of fertilization is expected to shoot up the crop production (Drostkar *et al.,* 2016). Large scale application of chemical fertilizers to increase the yield of crop is less favorable option as chemical fertilizers act as mixed blessings to the agricultural science as it increases the crop production but on long run, lead to slackening of the soil fertility and disturbs the mineral balance of the soil (De Oliveira *et al.,* 2020).Various technologies are being studied that can possibly contribute towards fulfilling the global food needs of continuously increasing global population, without disturbing soil sustainability. One such advancement in agricultural field is the application of nanotechnology (AL-Tameemi *et al.*, 2019).

Necessity of detailed study exists to understand the advantage and disadvantage of operation of nanoparticles and their environmental effects (Sangeetha *et al.,* 2017). The present day cropping system involves use of fertilizers which aids in increasing productivity. Micronutrient source particles of size of the order of 10^{-9} meter, named as nano fertilizers had shown to enhance the growth of vegetables and fruits on their application (Sangeetha *et al.,* 2017).

Absence of adequate amount of nutrients in soil acts as a resisting phenomenon in plant growth. Intensive cultivation also resulted in micro nutrient deficiency and is known to be one of the yield limiting factors (Kumar and Singh, 2016).Iron (Fe) is such a micronutrient which is required for respiration, photosynthesis and phytohormone synthesis in plants (Hansch and Mendel, 2009).

Nanoparticles of iron generally occur as oxides of magnetite nanoparticles (Fe_3O_4), Maghemite nanoparticles (g-Fe_2O_3), and hematite (α-Fe_2O_3), among others (Ali *et al.,* 2016). It is used in many applications owing to its inexpensive nature, yet waiting for extensive exploitation in the field of agriculture. Its non - toxic, accessibility and biocompatible properties have positioned iron in

recent times as one of the transition metal nanoparticles of current interest for improved agricultural production (Elemik *et al.,* 2019). Although it is a micronutrient, the lack of iron has shown abnormal growth in plants especially in the ones growing in alkaline soils. Many of the vital functions of plants, like enzyme, chlorophyll production, nitrogen fixation and development and metabolism depend on iron (Drostkar *et al.,* 2016). In comparison with common chemical fertilizers, nano-fertilizers have larger specific surface area, which makes nutrients easily available for absorption by plants and significantly improves its use. The application of nano-fertilizer can improve the physical and chemical properties of soil and improve water and fertilizer conservation (Teng *et al.,* 2018). Hochmuth (2011) reported that the annual requirement of iron in plants is 2 - 16 kg/hect are compared to nitrogen being 89-224 kg/hectare. Amongst the various methods, the foliar method of application of NP fertilizers has resulted in the enhancement in plant growth. It is also reported that supplementary foliar fertilization during crop growth can boost up the mineral status of plants and increase the crop growth and yield (Yildirim *et al.,* 2007).
The crop broccoli, classified as a variety of *Brassica Oleracea var. Italica*, belonging to the Cruciferae family is grown during winter-season for its green flowering head. It is closely related to cabbage, cauliflower, and kale and brussels sprout crops. Broccoli is the richest source of protein and vitamins among cole crops group. It also carries with it the reputation of a superfood. Although it is low in calories, it has a good content of anti-oxidants and nutrients. Being a rich source of sulforaphane and glucosinolates, it is expected to have anti-carcinogenic properties; eating more than one meal of broccoli a week reduces the risk of cancer by 45% and helps preventing heart disease (AL-Tameemi *et al.,* 2019).

Broccoli is a heavy fertilizer feeding crop that requires regular application of compound and micro nutrient fertilizer during the crop cycle. This study is carried out to determine impacts of stabilized Fe_3O_4 NP foliar application on head yield and iron content in broccoli head.

MATERIALS AND METHODS

The field experiment was conducted during the *Rabi* season of 2020 at the Research Farm, Fertilizer Nagar, Vadodara, Gujarat. The Fe_3O_4 NPs were synthesized in the laboratory and characterized using standard techniques. Seeds for Broccoli crop and compound fertilizer (Nitrogen, Phosphorus and Potassium), were purchased from commercial suppliers. $FeSO_4.7H_2O$ and Fe-EDTA (commercially available fertilizers as per norms of Fertilizer Control Order version 2019, Government of India) are procured and used.
Characteristics of the soil before crop transplanting (Table 1) were analyzed according to Page and Keeney(1982).

Field Experiment

The experiment was laid out in randomized block design comprising of five treatments *i.e.*, T_1– Fe EDTA, 300 ppm; T_2 – $FeSO_4.7H_2O$, 195 ppm; T_3 – EDTA stabilized Fe_3O_4 NPs, 6 ppm; T_4 - EDTA stabilized Fe_3O_4 NPs, 3 ppm and T_5 – Recommended dose of fertilizer (RDF). All the treatments are replicated thrice. Before sowing, till good tilth the field was thoroughly ploughed and leveled then manual raised-beds with 45 cm width and 25 cm height were prepared. Healthy broccoli seedlings were transplanted on 21.10.2020 at spacing of 60 × 60 cm row x row distance in the plot size of 4 m X 3 m (Length X Width) and harvested on 11.12.2020 to 02.01.2021. The recommended dose of fertilizer was incorporated into the soil of the respective plots up to a depth of 15 cm one day before transplantation. The four different concentrations *viz.*, Fe EDTA, 300 ppm, $FeSO_4.7H_2O$, 195 ppm and EDTA stabilized Fe_3O_4 NPs, 6 ppm and 3 ppm using three iron

Table 1: Pre-trial properties of experimental area soil

Organic carbon %	P_2O_5 kg/acre	K_2O kg/acre	pH	EC	S ppm	Zn, ppm	Fe, ppm	Mn, ppm	Cu, ppm
0.49	18	77	7.10	0.30	11.30	1.60	6.53	0.81	2.36

fertilizer sources was applied as foliar feeding to broccoli variety (cv. F_1 HYB NS-50). Spraying was performed in the morning (about 9.30 am), after the evaporation of the dewdrops. First spraying was done after 10 days of transplanting and subsequently 2nd at 30 days after transplanting with the help of hand sprayer. The plant height, stalk length, yield attributing parameters like fresh head weight, head girth and yield such as head yield and biological yield as well as harvest index along with NPK and iron (Fe) content were recorded during the experiment from the respective experimental plot by using standard procedures and expressed in standard units. Statistical analysis of the recorded data was carried out using analysis of variance technique for randomized block design (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Yield Parameters

The plant height data of broccoli at different stages of growth is presented in the Table 2.1. shows the significantly maximum plant height (53.33 cm) registered under foliar spray of EDTA stabilized Fe_3O_4 NPs with 3 ppm concentration over that of recommended dose of fertilizer (48.26 cm) but found statistically at par with foliar spray of conventional Fe EDTA solution with 300 ppm concentration (52.33 cm), $FeSo_4.7H_2O$ of 195 ppm concentration (52.40 cm) and EDTA stabilized Fe_3O_4 NPs with 6 ppm concentration (52.26 cm) at the time of harvest. Iron nutrient helps in construction of plant cells like cytochromes, phytofurthin and ferredoxins, which act as an electron transport in the process of photosynthesis, which contributes in vegetative growth of the plant (Chanwala *et al.,* 2019). When iron is applied in the form of nano particles, it results in increased head yield. Application of 100 times lower concentration of Fe as nano particles fertilizer (NFs) when compared to commercial Fe nutrient sources, yields significantly high yield of broccoli. Maximum stalk length is recorded with the foliar spray of EDTA stabilized Fe_3O_4 NPs with 3 ppm concentration which is significantly higher than those recorded for application of recommended dose of fertilizer, conventional Fe-EDTA with 300 ppm concentration and EDTA stabilized Fe_3O_4 NPs with 6 ppm concentration. It is worth noting that the best stalk length is at par with that recorded with application of 195 ppm solution of $FeSO_4.7H_2O$. The

Fig. 1: Broccoli head in response to different fertilizer

crop which received foliar spray of Fe_3O_4 NPs of 3 ppm concentration significantly increased the stalk length by the magnitude of 13.94 and 15.41 per cent at 40 days after transplanting (DATP) and at harvest respectively over recommended dose of fertilizer. Iron is important for the formation and activity of chlorophyll and in the functioning of several enzymes and the growth hormone like auxin. Importance of auxin in photosynthesis and other plant functions are extensively discussed by Kumari and Panigrahi (2019). Availability of auxin to the plant could have increased the inter-nodal length coupled with more apical dominance which might have helped for maximum stalk length and in turn resulted in higher vegetative growth, similar to the findings reported by Singh *et al.,* 2018.

Head girth values for all the treatments vary insignificantly (Table 1.). Although the highest value is recorded for the treatment is 195 ppm solution of $FeSO_4.7H_2O$, The fresh head weight data indicate that foliar application of Fe_3O_4 NPs with 3 ppm concentration resulted in significantly high (0.539 kg/plant) head weightas as compared to recommended dose of fertilizer. However, the value is found to be at par with that obtained with application of conventional Fe-EDTA with 300 ppm concentration and EDTA stabilized Fe_3O_4 NPs with 6 ppm concentration.

Head yield data is given in Table 1. Application of Fe_3O_4 NPs with 3 ppm concentration increaseshead yield (kg/plot and q/ha) and biological yield (q/ha) significantly over the application of recommended dose of fertilizer, conventional Fe EDTA with 300 ppm concentration and EDTA stabilized Fe_3O_4 NP with 6 ppm concentration. The treatment led to increase head and biological yield as compare to RDF (for EDTA stabilized Fe_3O_4 NPs (3 ppm)) by15.59 % and 9.82 % respectively, followed by that

Table 2.1: Effect of foliar spray of NPs fertilizer on growth and head yield attributes of broccoli cultivar.

Treatment	PH (cm) at harvest	SL (cm) at 40 DATP	SL (cm) at harvest	HG (cm)	FHW (kg Plant^{-1})	HY (kg/ plot)	HY (Q/ hectare)	BY (Q/ hectare)	HI (%)
T_1– Fe EDTA, 300 ppm	52.33	14.03	19.86	14.613	0.481	19.323	161.025	414.027	38.987
T_2– $FeSO_4.7H_2O$, 195 ppm	52.40	14.72	21.693	15.01	0.528	21.069	175.578	452.523	38.807
T_3 EDTA stabilized Fe_3O_4 NPs , 6 ppm	52.26	14.02	20.831	14.833	0.513	18.274	152.281	422.417	36.103
T_4 EDTA stabilized Fe_3O_4 NPs , 3 ppm	53.33	15.12	22.167	14.827	0.539	21.477	178.975	460.917	38.833
T_5– Recommended dose of fertilizer only	48.26	13.27	19.207	14.673	0.490	18.579	154.828	419.41	36.963
SEM±	0.74	0.26	0.584	0.211	0.008	0.594	4.948	10.804	1.332
CD at 5%	2.45	0.86	1.935	**	0.025	1.966	16.387	35.781	**
C.V. %	2.48	3.18	4.877	2.467	2.569	5.209	5.209	4.313	6.082

(PH: Plant Height; SL: Stalk Length; HG: Head Girth; FHW: Fresh Head weight; HY: Head yield; YQ: Yield quintal per hectare; BY: Biological Yield; HI: Harvest Index= [(total head yield)/(total biological yield)]100 ; DATP: Days after transplanting. '**' are non-significant at 5% level, respectively)

Table 2.2: Effect of foliar spray of NPs fertilizer application on N, P, K and Fe content in head dry matter of broccoli cultivar

Treatment	Nitrogen (% w/w)	P_2O_5(% w/w)	K_2O (% w/w)	Fe (mg/kg^{-1})
T_1– Fe EDTA, 300 ppm	5.54	1.60	1.82	204
T_2– $FeSO_4.7H_2O$, 195 ppm	5.64	1.62	2.31	284
T_3– Fe3O4 NPs , 6 ppm	5.62	1.61	2.49	259
T_4– Fe3O4 NPs , 3 ppm	5.53	1.4	2.47	512
T_5– Recommended dose of fertilizer only	5.58	1.6	2.60	228
SEM±	0.057	0.05	0.121	165.771
CD at 5%	**	**	0.401	**

'**' are non-significant at 5% level, respectively.

for $FeSO_4.7H_2O$ (195 ppm) which is 13.40 % and 7.89 % respectively). The recommended dose of fertilizer (RDF only) proved to be least effective in improving the head yield and biological yield as compared to EDTA stabilized Fe_3O_4 NP (3 ppm). The range of harvest index enhancement over recommended dose of fertilizer due to iron NPs fertilizer treatment was 36.10 % to 38.99 %. This result is in agreement with that reported by Jett *et al.,* 1995 in broccoli cultivar. Improvement in yield characters and yields as a result of foliar application of micronutrients might be due to the enhancement in photosynthesis and other metabolic activity which led to an increase in various plant metabolites responsible for cell division and elongation (Singh *et al.,* 2018).

Nutrient content

The maximum value of nitrogen content in broccoli heads was recorded with treatment $FeSO_4.7H_2O$,195 ppm (5.64 %) followed by the EDTA stabilized Fe_3O_4 NPs with 6 ppm concentration (5.62 %), which are at par with other treatments. The lowest nitrogen content was recorded with EDTA stabilized Fe_3O_4 NPs, with 3 ppm concentration (5.53 %). Nutrient content analysis of edible heads on dry basis reveal that nitrogen content ranged from 5.54 % to 5.64%. Phosphorus content ranged from 1.48 % to 1.64 % which is comparable to the P levels reported by Munro *et al.,* 1978. Potassium content was highest in the RDF treatment (2.60%) and lowest in treatment, Fe EDTA, 300 ppm (1.82 %).

The maximum value of iron content (512 mg/kg^{-1}) in head dry matter was recorded with treatment, Fe_3O_4 NP (3 ppm) followed by the $FeSO_4.7H_2O$ (195 ppm) (284 mg/kg^{-1}) and EDTA stabilized Fe_3O_4 NP (6 ppm) (259 mg/kg^{-1}). The lowest iron content (204 mg/kg^{-1}) was recorded with Fe EDTA (300 ppm).

Uptake of iron by plants is known to be influenced by other macromolecules. Judy *et al.*(2012) demonstrated that macromolecules could change NPs physicochemical properties and influence their uptakes and movements into rhizosphere and xylem sap. Zhu *et al.*(2008) showed that Fe_3O_4 NPs can be uptake by pumpkin (*Cucurbita mixta*) in fluid medium. Maynard (1979) reported that iron concentration of 100 ppm is sufficient for normal growth of vegetables. However, Purvis and Carolus (1964) reported that the iron content of normal plant tissue varied from 25 to 500 ppm, depending on parts of plant and their species. The variations in the assimilation of Fe from the nano-fertilizers at two different concentrations and their influences on other parameters - even though marginally significant - may be associated with influence of presence of other molecules.

CONCLUSION

Application of stabilized nanoparticles of Fe_3O_4, as a source of Fe micronutrient to broccoli has a positive influence on overall growth and yield. Significantly high values of height, stalk length, fresh head weight, head yield and biological yield indicate that application of these nanoparticles at a very low concentration is considerably effective. With the application of a higher concentration of the nanoparticles of iron, differences in the results are insignificant, which indicates the wastage of nutrients and fertilizer. Major nutrient contents (NPK) in the edible heads remain normal with the application of nanoparticles as fertilizers.

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