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Role of micronutrients on morpho-physiological, and nutritional parameters including diseases suppression of fenugreek

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Abstract

Micronutrients play a crucial role in enzymatic activities of plant and ultimately growth, yield and quality. Present investigation was carried out with five micronutrients viz. Fe_{-10}, Zn_{-05}, Mn_{-10}, Cu_{-05}, B_{-05} and their effect was compared with control for growth, yield, diseases, nutritional and physiological parameters of fenugreek (*Trigonella foenum graecum*). Growth parameters like plant height, number of pods, root nodulation were improved by these nutrients. However, seed germination was hastened by Fe, Zn, Mn and Cu, whereas B delayed the germination for more than a day. Seed yield was significantly higher with Fe and Mn and marginally with Zn, Cu and B whereas haulm yield was more with all the elements. The per cent yield increased with Fe and Mn was 18.9 and 20.0, respectively. Uptake of N, K, Cu, B and Mn enhanced by most of the applied micronutrients. However, P uptake was only higher with Cu and B, while Fe with B and Fe. Likewise, Zn uptake was more with application of B, Cu and Zn. Chlorophyll, a, b, total, carotenoids and soil available nutrients were marginally influenced by application of these micronutrients. Crop maturity was advanced by all the micronutrients, while the earliest maturity was noticed with B, which was advanced for more than a week in comparison to control. Based on the Percent Disease Index, fenugreek blight was suppressed by all the micronutrients very effectively, whereas powdery mildew was only lower with Mn. However, soil application of Mn, Cu, Zn and B were more effective for the control of downy mildew.Overall effect of Fe and Mn was superior among all the applied micronutrients, whereas role of B was magical for delaying seed germination and hastening maturity.

Key words: Blight, downy mildew, fenugreek, growth, nutrient uptake, powdery mildew, soil available nutrients.

Introduction

Micronutrients are essential elements that are used by plants in small quantities. Yield and quality of agricultural products increased with micronutrients application, therefore human and animal health is protected with feed of enrichment plant materials. Each essential element can perform its role in plant nutrition properly when other necessary elements are available in balanced ratios for plant (Tavakoli, *et al.*, 2014). Copper is absorbed in the form Cu^{+2}. It is contributory with enzymes in oxidation reactions, an example of such enzyme is plastocyanin, which is associated in photosynthesis by transfer of electron in light reactions (Haehnel, 1984), but its availability in soil is pH dependent. In fact, the amount of seeds in plants is related to the amount absorbed by these elements (Loomis & Conner, 1992). Iron has an essential role as an involved enzyme components in electrons transfer and cytochromes. It is reflective oxidizes from Fe^{+2} to Fe^{+3} during electron transfer (Bienfait & Van der Mark, 1993). Iron is necessary for the synthesis of chlorophyll and plays a crucial role in ferredoxin, flavoprotien and respiration (Verma, 2007). Boron plays main roles in elongation of cell, synthesis of nucleic acid, hormone responses and function of membrane (Shelp, 1993). Many enzymes require manganese ions for their activity in plant cells. e.g., dehydrogenases and decarboxylases involved in the Krebs cycle are activated by manganese. The best role of manganese is the transfer of electrons reaction in which oxygen is produced from water. It plays role in the synthesis of chlorophyll and the electrons transfer from H_2O to photo- oxidized chlorophyll in photosynthesis (Marschner, 1995). Zinc function as an enzyme activator in some reactions, e.g., carbonic anhydrase, hexose kinase and alcohol dehydrogenase. Zinc is important for the biosynthesis of the indole-3-acetic acid. It is believed

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to be concerned with protein metabolism and photosynthesis (Verma, 2007). Zinc and Fe deficiency can be seen in eroded, arid alkaline/calcareous and weathering acidic soils. Boron and manganese deficiency symptoms appear in spring under cool and wet conditions due to reduced roots metabolic activity and uptake. Biochemical functions of micronutrients in crop plants translate in improving yield, quality and anatomical structures of fenugreek cv. Giza 3 (Boghdady, 2017) in Egypt. There were some studies made on micronutrients for fenugreek production either with one or two elements, like Fe by Chhibba *et al.*, (2007), B (Pariari, *et al.*, 2009), Mo (Sharma *et al.*, 2014) and Zn (Kumawat *et al.*, 2015; Lal *et al.*, 2015) and mixture as foliar fertilizer (Amal *et al.*, 2015). Hence, there is no single study available to address the comparative response of various micronutrients as a limiting factor for fenugreek. Therefore, present investigation was carried out with B, Cu, Fe, Mn, and Zn as soil application.

Materials and methods

Location and climate

The field experiments were carried out for two consecutive years under the Typic Haplustepts during Rabi season of 2014–2015 and 2015–2016 at ICAR-National Research Centre on Seed Spices, Tabiji, Ajmer, Rajasthan, India. This was laid out between 74° 35'39” to 74° 36' 01”E longitude and 26° 22'12” to 26° 22' 31” N latitude. Climate of the Ajmer area characterized as semi-arid. The average annual rainfall of the area is 536 mm and most of it (85-90%) received between June to September. July and August are most rainy months contributing 60.0% of the average rainfall. The moisture control section remains dry for more than 90 cumulative days and hence moisture regime classified as Ustic. The mean annual temperature is 24.5 to 25.0°C. January is the coolest month of the season and temperature remain around 7.0°C. Currently frost is also occurring in this month with changing climatic pattern (Singh and Shyampura, 2004).

Treatments and cultural practices

The treatments consisted Fe-10, Zn-05, Mn-10, Cu-05, B-05 and compared with control. The subscript values given with nutrients is the amount of those nutrient applied in the soil (kg ha^{-1}). Nutrients amount for the treatments was decided based on the laboratory experiments for germination and soil availability in the experimental field. Sulphates of Fe, Zn, Mn and Cu were taken as fertilizer and B as boric acid. All the six treatments were arranged in a Randomized Block Design (RBD) with four replications. The fenugreek varieties AFg-1 was taken as a test crop for the study on sandy loam soil. Seeds of the fenugreek variety AFg-1 were sown during winter season and plant spacing 25cm line to line apart and from plant to plant distance was maintained at 10 cm by thinning. Cultural practices were uniformly followed during the growing seasons in both the years and crop was irrigated as and when required. The crop was harvested and seeds were separated from the haulm by beating bundles thereafter winnowing.

Soil analysis

Soil samples were collected from the surface (0-15 cm depth) before sowing of seed. Samples were air dried and powdered with wooden mortar and pestle and passed through a 2 mm stainless steel sieve. Experimental soil was analysed for physicochemical properties i.e. EC and pH (Richards, 1954), organic carbon content by rapid chromic titration (Walkley and Black, 1934), available N by alkaline permanganate (Subbiah and Asija, 1956), available P by 0.5 M $NaHCO_3$ extractable P (Olsen, *et al.*, 1954; Bray and Kurtz 1945), available K by 1N NH_4OAc extracts method (Jackson, 1973) and available micro-nutrients (Fe, Zn, Mn & Cu) by DTPA (Lindsay, and Norvell, 1978) and boron by hot water extraction and Azomethine-H, colorimetric method (Jackson, 1973 & Capelle, 1961) after standardization.

Texture of experimental soil was sandy loam. Soil EC, pH and organic carbon were 0.31 dSm^{-1}, 8.6 and 0.21%, respectively. However, soil available N, P and K were 90.1, 7.8 and 350 kg ha^{-1}, respectively. Micronutrients status like iron, zinc, manganese, copper and boron in the soil was 12.5, 2.5, 17.5, 2.5 and 3.5 kg ha^{-1}, respectively. Soil calcium content was about 7.5 per cent.

Plant analysis

The plant samples were collected after the harvest of crop. These samples were successively washed with tape water and then 0.1 M HCl followed by distilled water and thereafter dried at 70°C. After proper drying samples were powdered in wily mill and passed through the 20 mesh stainless steal sieve. Nitrogen was estimated by Kjeldahl method (Piper, 1966). The samples were digested in nitric and perchloric acid (10:4) for the estimation of P by Venado-molybdo yellow colour method (Chapman, and Pratt, 1962) and K by flame photometer. Iron, zinc, manganese, and copper were estimated by Atomic Absorption Spectrophotometer and B by Azomethine-H, colorimetric method (Capelle, 1961), which was modified as per our conditions.

Statistical Analysis

The data of both the years were analyzed by ANOVA and treatment differences were expressed for Least Significant differences (LSD) at 5% probability to determine the significance among the treatment means (Cochran and Cox, 1987).

Results and discussion

Growth parameters at 50 days

Growth parameters were recorded at the age of 50 days and it was found that seed germination was hastened by Fe and marginally by Zn, Mn and Cu, however the longest delayed for more than a day by boron (Table 1). Distinctiveness was also confirmed by lab experiments for germination, where gap was more distinctive than field level. Root area was more only with Fe and remained lower with all the other micronutrients. This might be due to the fact that under micronutrient stress, plant expands the more root surface area so as to absorb required amount of nutrient to fulfil the plant need. Root diameter was inversely related to the area with applied nutrients and it is obvious. Total root and shoot length at this stage and number of leaves per plant were higher with Fe, Zn and Mn, while tape root was only higher with Fe and Mn. In contrast to these parameters number of root nodules per plant were more with all the applied micronutrients. However, highest root nodulation was with Fe followed by Mn and B. This is because of crop responded to these nutrients at the age of 50 days. Chlorophyll a, b, total and carotenoid content was marginally higher with all the nutrients except B (Fig.1). Improvement in above growth and physiological parameters are warranted as micronutrients serve many roles in plant metabolism and growth. One of the frequent functions of micronutrients is to serve as catalysts or co-factors of various enzymes system involved in different metabolic reactions resultant higher morphological and physiological parameters (Dutta *et al.*, 2017).

Growth and yield parameters at maturity

Most of the growth parameters were marginally higher with these nutrients, however, plant height and number of pods per plants were higher statistically (Table 2). This distinctiveness among the treatments reduced at maturity in comparison to 50 days may be due to fixation of micronutrients and reduced availability with time at higher soil pH. Plants were tallest with B and Mn and pods were the highest in number with Mn and Zn, though

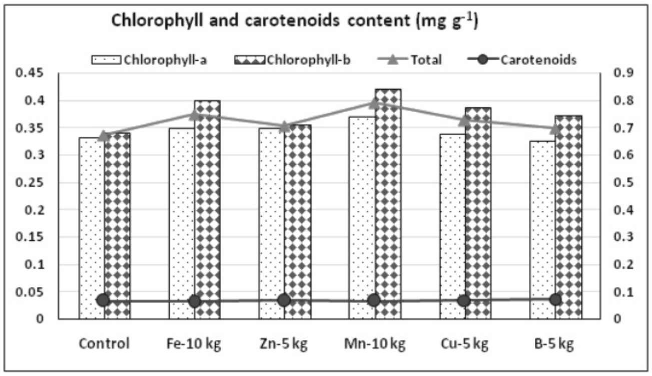

Fig. 1. Chlorophyll content in leaves at 55 days in response to soil application of micronutrients

Table 1. Root and shoot parameters of fenugreek at 50 days in response to soil application of micronutrients

Treatment	Seed germination (hrs)	Root area (mm^2)	Root diameter (mm)	Total root length (mm)	Shoot length (cm)	Taproot length (cm)	No of nodule $plant^{-1}$	No of leaves $plant^{-1}$
Control	76.3	403.6	1.12	327.0	14.6	8.3	7.1	6.0
Fe-10	70.5	486.6	1.16	372.9	16.6	9.2	9.3	6.6
Zn-05	72.5	324.9	1.26	366.4	15.8	9.2	8.4	6.8
Mn-10	73.2	318.1	1.28	288.1	16.6	8.7	8.8	6.7
Cu-05	75.5	336.7	1.24	321.2	15.5	8.6	8.5	6.2
B-05	95.5	390.0	1.28	340.8	15.4	8.9	8.8	6.4
LSD at 5%	5.7	69.40	0.09	29.2	0.9	0.8	0.7	0.5

Values in parenthesis in kg ha^{-1}.

Table 2. Growth parameters of fenugreek at maturity with soil application of micro-nutrients

Treatment	Plant Height	Number of				Pod length (cm)	Days to maturity	Yield (q ha^{-1})	
		PB	SB	Pod Plant^{-1}	Seed pod^{-1}			Seed	Haulm
Control	81.5	6.0	5.1	27.5	20.6	11.3	132.0	17.5	33.0
Fe_{10}	83.6	6.5	5.9	28.9	21.1	11.8	130.2	20.8	37.5
Zn_5	81.8	6.2	5.9	29.8	20.9	11.7	130.1	18.2	36.6
Mn_{10}	84.5	6.4	5.8	29.8	20.9	11.8	129.4	21.0	37.3
Cu_5	82.6	6.3	5.2	28.8	21.8	11.9	127.3	18.0	35.6
B_5	84.6	6.4	5.3	29.0	21.9	11.9	125.5	18.7	35.7
LSD at 5%	2.0	NS	NS	1.3	NS	NS	5.3	2.07	2.51

The treatment values given in subscripts are in kg ha^{-1}. PB:Primary Branches, SB, Secondary Branches

pods were more with all the applied micronutrients as compared to control. It indicates that micronutrients have greater impact on plant height and pod numbers than any other parameters. Foliar application of B and Zn twice is most effective for more plant height, branches and pods of fenugreek (Pariari, *et al.*, 2009) and by 0.5% Zn (Lal *et al.*, 2015). Effect of Fetril on Combi-2,(a micronutrient liquid mixture) reported useful for improving the productivity and growth of fenugreek (Giza-3) in Egypt (Amal *et al.*, 2015). Improvement in morphological and anatomical parameters by foliar application of micronutrients in cv Giza-3 was also observed in Egypt (Boghdady 2017). Likewise, it is very interestingly to note that micronutrients hastens the maturity in fenugreek, however role of B was prime in this regards followed by Cu and Mn. This indicates that balance nutrition is also very important for crop maturity. Many morphological impairment and delayed maturity in With ania somnifera was observed by application of graded levels of major nutrient could be balanced by micronutrient application (Aishwath, 2004). Liming improves the availability of micronutrients in acidic soil resultant improvement in fenugreek yield (Aishwath, *et al.*, 2016). In contrast to above findings delayed in 50% flowering by soil application of 30 kg $ZnSO_4$ ha^{-1}was also reported earlier (Kumawat *et al.*, 2015).

Seed yield was significantly higher with Fe and Mn and marginally higher with Zn, Cu and B (Table 2). The per cent yield increased with Fe and Mn was 18.9 and 20.0, respectively. In general, availability of micronutrients was medium to higher level in experimental soil, even then fenugreek responded well to Fe and Mn, and also marginally with other nutrients. Besides the growth parameters, higher seed and stover yield, net return and B:C ratio with 30 kg $ZnSO_4$ ha^{-1} was recorded by Kumawat *et al.* ,(2015). Higher green and seed yield of fenugreek was also reported by Chhibba *et al.*, (2007) with foliar application of Fe in deficient soils of these nutrients. Though the crop responded significantly to soil as well as the foliar application of Fe, yet the foliar mode proved significantly superior to soil application. Another study on B (0.1%) and Zn (0.2%) revealed that foliar application twice during crop growth was most effective for yield attributes and seed yield of fenugreek (Pariari *et al.*, 2009). Though the availability of Fe and Mn was highest in soil among the applied micronutrients yet there need of crop was further more. Haulm yield was higher along with all the micronutrients, however highest haulm yield was with Mn and Fe in comparison to control.

Nutrient uptake by fenugreek

Uptake of N was highest with Mn followed by Fe, Cu and B might be due to higher nutrient content and higher accumulation of biomass and seed yield (Fig. 2). Phosphorus uptake was only higher with Cu and B, might be due to the fact that Fe, Zn and Mn may have antagonistic effect on absorption and accumulation of phosphorus in both seed and haulm. Potassium uptake was higher with all micronutrients, however uptake was highest with Mn might be due to more seed yield and biomass accumulation. Uptake of Fe, Mn and B was also higher with their aloof input in the soil (Fig. 3). Iron content and uptake in fenugreek was improved by soil and foliar application of Fe in deficient soils for Fe (Chhibba *et al.*, 2007). Uptake of Zn was only higher with applied Zn, B and Cu, while uptake of B was higher with all the micronutrients except Zn might be due to antagonistic effect with each other on absorption by plant. Effect of Fetrilon Combi-2 (a micronutrient liquid mixture) reported useful for improving nutrient content and uptake of fenugreek in Egypt (Amal *et al.*, 2015).

Zinc uptake from soil solution in divalent cations form (Zn^{+2}) in calcareous soils with high pH, zinc uptake may be a valence ion form (Tavakoli, *et al.*, 2014). Among the major nutrients, uptake of N was highest followed by K, and P uptake was least. Uptake of Na was also in equal amount as that of N, however, its uptake was negatively influenced by applied micronutrients, except B. Hence, it can be inferred that application of micronutrients could mitigate the adverse effect of Na by hindering in its uptake by the crop. As in quantitative values, uptake of Fe was the highest followed by B and their uptake values were in kilograms, however uptake of Mn, Zn and Cu was in grams among the micronutrients.

Soil available nutrient after fenugreek

Macronutrients availability was marginally improved with application of all the micronutrients might be due to the higher root activity and nodulation by rhizobacteria (Fig.4). Experimental soil was slightly sodic in nature hence exchangeable Na in soil was worked out which was marginally lower with applied micronutrients, this because of sulphates of micronutrients applied in soil exchanged the Na from the soil micelle resultant lower exchangeable Na. Availability of all the micronutrients was marginally higher except B, where their salts were applied, however soil available B was significantly higher with applied boron only (Fig. 5). Availability of these nutrients was marginally lower than control. Possible reason for the lower availability in soil may be due to higher removal by crop and fixation in soil due to higher soil pH and lime content (7.5%). Zinc deficiency can be seen in eroded, calcareous and weathered acidic soils. Zinc deficiency is often accompanied with iron deficiency in calcareous soils. Iron in the soil is the fourth abundant element on earth, but its amount was low or not available for the plants and microorganisms needs, due to low solubility of minerals containing iron in many places of the world, especially in arid regions with alkaline soils pH. Boron and manganese deficiency symptoms that appear in spring under cool and wet conditions due to reduced roots metabolic activity and uptake tend to go away when soil conditions become warm and drier. Copper is immobile in soil, its solubility and plant availability is highly dependent on soil pH. Copper

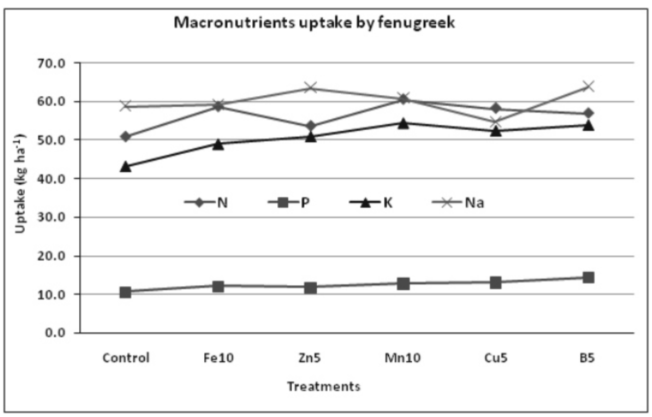

Fig. 2. Macronutrients uptake by fenugreek with soil applied micronutrients.

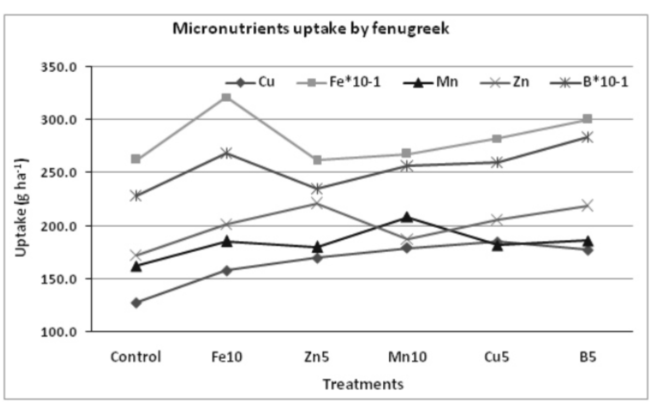

Fig. 3. Micronutrients uptake by fenugreek with soil applied micronutrients.

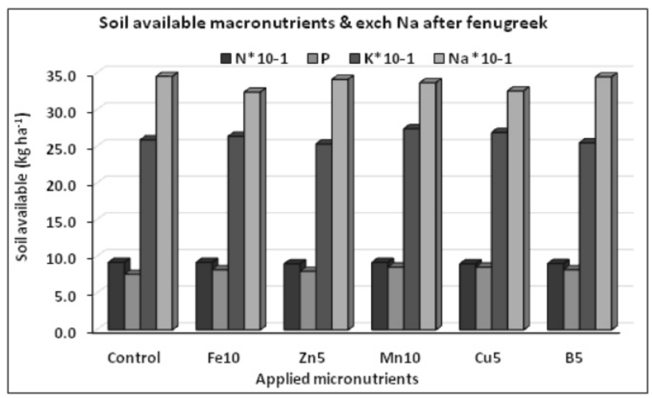

Fig. 4. Soil available macronutrients after fenugreek crops with soil applied micronutrients.

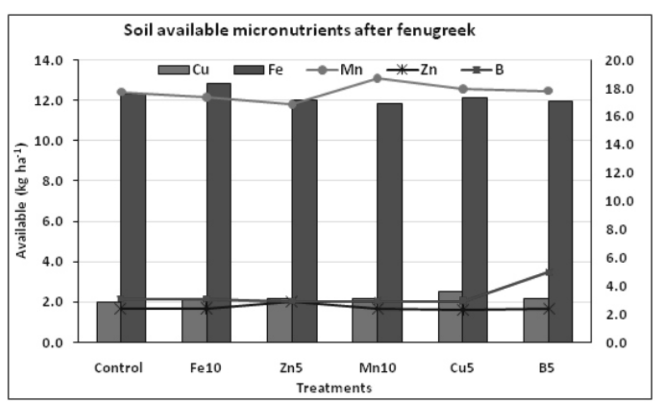

Fig. 5. Soil available micronutrients after fenugreek crops with soil applied micronutrients.

solubility increases approximately 100 fold for each unit decrease in soil pH Tavakoli *et al.*, (2014). Application of 20.0 kg each of Zn and Fe sulphate and 2.0 kg borax in sunflower under Inceptisol enhanced the availability of these nutrients in soil (Kumbhar *et al.*, 2017).

Per cent disease index (PDI) with applied micronutrients

The effect of micronutrients on reducing the severity of diseases can be attributed to the involvement in physiology and biochemistry of the plant, as many of the essential micronutrients are involved in many processes that can affect the response of plants to pathogens (Marschner, 1995; Dutta *et al.*, 2017). There were three diseases downy mildew, powdery mildew and blight appeared during the growth of fenugreek. Downy mildew was significantly suppressed by Mn application and marginally by Zn, Cu and B (Fig. 6). However, powdery mildew was only suppressed by soil application of Mn. Role of Mn in suppression of powdery mildew in fenugreek was reported earlier by Aishwath *et al.*, (2016) and Brain & Whittington (2007) in other crops. In case of blight, it was lower with all the micronutrients, however least PDI was observed with Mn. The Mn, Cu and B can suppress diseases by releasing Ca from cell walls through cation exchange, which interact with salicylic acid and activate systemic acquired resistance mechanisms (Reuveni *et al.*, 1997; Reuveni and Reuveni, 1998). Among the micronutrients, Mn can control a number of diseases as Mn has an important role in lignin biosynthesis, phenol biosynthesis, photosynthesis and several other functions. Besides that fungus of downy mildew required lesser amount of Mn for its cellular function than the plants resultant uncongenial cell environment suppresses the fungal disease. Mn inhibits the induction of amino peptidase, an enzyme which supplies essential amino acids for fungal growth and pectin methyl esterase, a fungal enzyme that degrades host cell walls (Dordas, 2008).

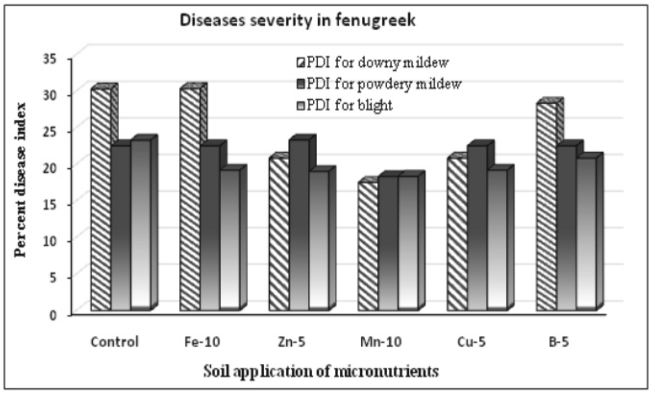

Fig.6. Severity of diseases in response to soil application of micronutrients

Conclusion

Based on the above findings, it can be concluded that fenugreek responds to soil applied micronutrients and most of it for Fe and Mn with respect to growth, yield and their parameters, changes in nutrient uptake and their availability in soil and suppression of diseases occurred. Role of boron was perceived unique for delaying seed germination and advancing in maturity. This indicates that balance nutrition is also very important for optimizing crop maturity.

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