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Bioactivation of Indigenous and Exogenously Applied Micronutrients through Acidified Organic Amendment for Improving Yield and Biofortification of Maize in Calcareous Soil

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Abstract

Micronutrients are required for the proper growth and development of plants, animals and human-beings. Unfortunately, owed to calcareous nature of Pakistani soils plants are suffering from severe micronutrients deficiency and to overwhelm this issue, various strategies have been adopted. Among these, elemental sulfur (Sº) is becoming a commonly practiced strategy, but, still it is accompanied by economic constraints because huge amount of Sº could not be paid by farmers. In the present study, sulfur oxidizing bacteria (SOB) were isolated and screened on the basis of pH reduction and sulphate ions production. The most efficient SOB strain was used to prepare acidified-amendment through biological-oxidation of S° amended cow-manure. After preparation and rate optimization of acidulated-amendment (liquid) in laboratory conditions, it was applied to pot grown maize (Hycorn) along with 25, 50, 75, 100% of the recommended dose of $ZnSO_4$, $FeSO_4$ and $MnSO_4$; to evaluate its impact on growth, yield and bioavailability of micronutrients (Zn, Fe and Mn). Results indicated that combined use of acidulated-extract with micronutrient significantly enhanced the growth, yield and micronutrient availability of maize as compared to control. However, on the basis of economics, 50% of the recommended micronutrient + acidulated extract was found more beneficial and improved yield by 46.27% and grain Zn, Fe and Mn concentration by 42.95, 117.38 and 128.55% over control, respectively. The results may imply that acidified-product can improve the grain micronutrient contents and resultantly, humans' micronutrient deficiency would be uplifted, proving it to be a novel and eco-friendly approach for calcareous soil.

Keywords: Acidulated organic amendment; Elemental sulfur oxidation; Acidified extract; Micronutrients; pH manipulation

Introduction

In developing countries, population density is increasing rapidly and demanding amplified agricultural production that is directly linked with soil nutrients availability (United Nations, 2012). So, fertility management is critical for optimization of crop nutrition to achieve the goal of sustainable crop production. Nutrient deficiency due to calcareousness is most serious and difficult issue to ameliorate globally (Nikolic *et al.*, 2016). It is the major problem of about 30% earth's culturable land and more than 80% soils of Pakistan due to higher quantity of free calcium carbonate ($CaCO_3$) (in Punjab >3%) and pH greater than 7, typically 7.5 to 8.5. Equally, malnutrition due to micronutrients deficiency is also a very popular issue of developing countries (Mayer *et al.*, 2008; Karimizarchi *et al.*, 2014) i.e., India, Nepal, Srilanka, Nigeria and Pakistan etc. The main reason considered behind this issue is also calcareousness, as due to high concentration of calcium ions, micronutrients such as iron, zinc, manganese etc. are fixed/precipitated (Alloway, 2008; Imran *et al.*, 2014) and their availability to the plants gets reduced significantly (Heydarnezhad *et al.*, 2012) except Mo.

Approximately, 40% of world's population suffers from micronutrient (Fe, Zn, Cu and Mn) deficiencies (the so-called "hidden hunger"), which causes severe yield reduction in plants and cause many disorders in human beings. Micronutrients play an active role in plant development, growth, metabolic processes; cell wall development to respiration; photosynthesis, chlorophyll formation, enzyme activity, nitrogen fixation and reduction. Additionally, they are activators of many enzymes and play essential role in crop yield and quality (Abbas *et al.*, 2012). So, deficiency of just one of these micronutrients can severally affect the plant growth, yield and even cause plant death (Liu *et al.*, 2006; Hao *et al.*, 2007). In modern age, with increasing population, world food demand is also increased (Borlaug, 1983) which leads to introduction of new high yielding and nutrient exhaustive varieties which drastically enhance the cereals yield but of low quality. This low quality food is mainly due to nutrient deficiency, especially micronutrients (Welch, 2002) and consumption of this food cause serious health consequences in human (inflated economic costs borne by society and learning disabilities for children) (Sanchez and Swaminathan, 2005; Hao *et al.*, 2007) due to their fundamental role in metabolism, maintenance of tissue function, role in cofactors in metabolism, coenzymes in metabolic functions (like riboflavin and niacin in the electron transport chain and folic acid as part of methyl group transfer), involved in genetic control (zinc ''fingers'') and in defense system.

For this reason, it is necessary to resolve this problem which affects every third child of our country. In literature, a number of strategies as breeding and modern biotechnology (to increase the micronutrient in crops) (Bouis *et al.*, 1999), addition of micronutrients to foods, condiments or beverages (Anonymous, 2007), providing micronutrients through tablets and syrups (Strand, 2003), and use of micronutrients containing fertilizers (Imtiaz *et al.*, 2006) are discussed but due to economic issues and nature of our soil (having more $CaCO_3$>3%, less organic matter and high soil pH) cannot be practiced successfully. By keeping in view the importance of micronutrients and previously employed strategies for correction of calcareous nature of soil, an acidified amendment was prepared through bio-augmentation of Sº amended cow dung with SOB. The acidified organic amendment along with different rates of micronutrient was evaluated to enhance growth, yield and micronutrient bioavailability of maize in calcareous soil.

Materials and Methods

A pot experiment was conducted in the wire house of the Institute of Soil and Environmental Sciences (ISES), University of Agriculture, Faisalabad– Pakistan, to evaluate the combined effect of acidified manure (extract) and different levels of micronutrients on growth, yield and micronutrients concentration in grains of maize. Soil was prepared (air dried, ground and after passing through 2 mm sieve, mixed thoroughly) and filled in 20 kg pots. Soil was collected from the research area, ISES, UAF and analyzed for various physico-chemical properties. The soil was sandy clay loam (sand 52.33, clay 21.45, silt 26.22%), organic matter 0.77%, EC 1.89 dS m^{-1}, pH 7.93, total nitrogen 0.085%, available phosphorus 4.5 mg kg^{-1}, extractable potassium 82 mg kg^{-1} and iron 4.4 mg kg^{-1}, zinc 0.51 mg kg^{-1} and manganese 0.43 mg kg^{-1}.

Preparation of Acidified Extract

Sulfur oxidizing bacterial strains were isolated from different ecologies and most efficient strains on the basis of pH lowering and SO_4^{-2} ions production in liquid broth were selected. Then, acidified product was prepared through bio-augmentation of Sº added cow dung with SOBs. Different rates of Sº were mixed with cow dung and after incubation period the most suitable formulation with minimum resultant pH was selected for further studies. Prepared product was optimized under different conditions for preparation of minimum pH product and at15thday of incubation, amendment of 2.1 pH was obtained at 35ºC temperature, 65-70% moisture, 0.35% molasses in presence of efficient SOB strain AR-13 (*Pseudomonas aeruginosa*). Prepared solid product was then diluted to different ratios and most economical dilutions (5, 10 and 15%) on the basis of minimum pH was selected and applied in soil with irrigation water under controlled conditions to determine micronutrient release pattern. Then, on the basis of micronutrient release pattern in soil, 10% dilution of solid acidified product was selected for further evaluation to enhance growth, yield and biofortification of maize under pot condition.

Experimental Design

A pot trial was conducted to evaluate the effect of acidified organic amendment for enhancing growth, yield and micronutrient uptake in maize. Five seeds of maize variety hycorn (ICI, Pvt. Ltd., Pakistan) were sown in each pot have 20 kg soil. Recommended dose of NPK fertilizers at the rate of 175-120-90 kg ha^{-1} were applied as urea, diammonium phosphate (DAP) and sulphate of potash (SOP). Micronutrient Fe, Zn and Mn were applied at the rate of 25, 50, 75 and 100% of the recommended dose (15, 12, 5 kg ha^{-1}) as $FeSO_4$, $ZnSO_4$ and $MnSO_4$, respectively. After germination one plant per pot was maintained and treatments were arranged according to completely randomized design (CRD) with three replications of each. The acidified extract (AE) was applied at four critical stages at the rate of 247 liters/ha with fertigation. Tap water was used for irrigation. Maize was harvested at maturity and data regarding growth (plant height, fresh shoot biomass, dry shoot biomass), yield (cob growth parameters, 100 grains weight) and micronutrients contents in plant and release in soil were recorded.

Measurement of Micronutrients in Root, Shoot and Grains of Maize

Air dried ground material (root, shoot and grain) (1 g) was placed in the digestion flasks. Ten mL of reagent (nitric acid and perchloric acid as 2:1 ratio) was added into it and left overnight, next day samples were heated on the hot plate till the production of red NO_2 fumes were ceased. The flasks were allowed to cool down and then added a small amount (2–4 mL) of 70% $HClO_4$, the samples were heated again and allowed evaporating to a small volume. When the vapors were condensed, contents of the flask were transferred to 50 mL volumetric flask and volume was made with distilled water. Each batch of digestion samples contained at least one reagent blank (no plant material).

Samples were then filtered and used for determination of Zn, Fe and Mn by atomic absorption spectrometry.

Measurement of Micronutrients Zn, Fe and Mn in Soil

Micronutrients Zn, Fe and Mn were determined by ammonium bicarbonate-DTPA extractable method developed by Soltanpour and Schwab (1977), and later modified by Soltanpour and Workman (1979). For this, 10 g air dried soil (2 mm) was taken into a 125 mL conical flask. Twenty mL of extracting solution was added and shaken on a reciprocal shaker for 15 min at 180 cycles/min with flasks kept open. The extracting solution was 1 M ammonium bicarbonate (NH_4HCO_3), and 0.005 M DTPA adjusted to pH 7.6. The extracts were then filtered through Whatman No. 42 filter paper. Zinc, Fe and Mn were determined by atomic absorption spectrophotometer.

Statistical Analysis

The data were analyzed using analysis of variance technique (ANOVA) with CRD design (Steel *et al.*, 1997). For this purpose, computer software Statistix 8.1 was used and means were compared by least significant difference (LSD) test.

Results

Plant Growth Parameters

Application of micronutrients + acidified extract significantly enhanced the vegetative growth parameters i.e. plant height, plant fresh and dry biomass, leaf length and leaf width of maize (Table 1) as compared to control. Although, acidified extract + micronutrients (100, 75, 50 and 25% of recommended dose) treated soil showed highest vegetative growth compared to others but where 100, 75 and 50% of the recommended dose of micronutrients was applied with acidified extract showed higher but almost similar outcomes. In comparison with alone application of micronutrients, 9.81, 8.72 and 8.71% (fresh weight), 23.46, 19.55, 21.50% (dry weight) and 9, 8.61 and 7.87% (plant height) increment was recorded in case of 100, 75 and 50% micronutrient + acidified extract treated soil, respectively. Same trend was recorded from leaf length and width related parameters. But in comparison with control (no added micronutrients), treatment where micronutrients alone were applied, showed 31.45% increase in fresh weight, 36.15% increase in dry biomass, 30.95% increase in plant height, 37.25% increase in leaf length and 23.52% increase in stem diameter.

Yield Related Parameters

Cob growth parameters: Integration of micronutrients and acidified extract tend to improve the cob growth parameters of maize (Table 2). Maximum increase in cob growth parameters were recorded where acidified extract was applied along with different rates of micronutrient compared to sole application of micronutrients. In case of grain/cob maximum results, 319 grain/cob, 325 grain/cob and 335 grain/cob were recorded from 100, 75 and 50% of the recommended dose of micronutrients + acidified extract, respectively, and if we compare these values with recommended dose of micronutrients then 72.43, 75.76 and 81.08% increment, was recorded from these, respectively. Stover yield also showed almost similar trend; maximum stover yield recorded was 35.98, 35.78 and 35.77 g from 100, 75 and 50% of the recommended dose of micronutrients + acidified extract, respectively and in comparison with recommended dose of micronutrients 27.99, 27.28 and 27.25% increment was recorded from these above said treatments, respectively. Almost same trend was recorded from cob diameter, cob length and grain/line; treatments with 100, 75 and 50% of the recommended dose of micronutrients + acidified extract (pH 2.2) showed highest increase which is followed by treatments where 25% micronutrients with acidified extract were applied. Separate application of micronutrients and acidified extract differed non-significantly from each other; however, both showed significant increase in cob growth parameters in comparison with control.

100 Grains Weight

The data in Fig. 1 revealed that 100 grains weight was also significantly influenced by the application of micronutrients + extract compared to sole micronutrients and control. Alone application of micronutrients and extract increased the grain yield up to 16.66 and 22.22% compared to control, respectively. Maximum increase in grain yield was recorded were extract was applied with 100, 75 and 50% of the recommended dose of micronutrients, followed by treatment where 25% of recommended dose of micronutrients and extract was applied. Maximum 100 grain weight of 27, 25.67 and 26.33 g were obtained from treatments where 100, 75 and 50% of recommended dose of micronutrients + acidified extract were applied, respectively and in comparison with recommended dose of micronutrients these results showed 22.7, 16.68 and 19.68% and in comparison with control showed 50.01, 42.61 and 46.27% increase, respectively. Treatments with acidified extract and only micronutrients showed almost same results; while control, where no micronutrients were applied showed minimum grains weight of 18 g/pot only.

Micronutrients Concentration in Plants Tissues

Zn concentration in shoot, root and grains of maize: Data regarding the effect of micronutrients with and without acidified extract on Zn concentration in shoot, root and grains of maize are presented in Table 3. The micronutrients containing treatments i.e., 100, 75, 50

Table 1: Effect of acidified extract on plant weight, leaf width, leaf length and stem diameter of maize

Treatment	Plant fresh weight (g)	Plant dry weight (g)	Plant height (cm)	Leaf width (cm)	Leaf length (cm)	Stem diameter (cm)
Control (NPK)	181.9f	26.0d	135.7d	5.1d	73.0 d	3.4 e
10% AE + NPK	209.9e	30.7c	177.0c	6.3 c	81.0 c	4.0 d
Micronutrients + NPK	238.3c	35.4b	177.7c	7.0 b	80.7 c	4.2 c
10% AE + 100% Micronutrients + NPK	261.7a	44.2a	193.7a	7.7 a	93.0 a	5.1 a
10% AE + 75% Micronutrients + NPK	259.1b	42.6a	193.0a	7.6 a	93.0 a	5.1 a
10% AE + 50% Micronutrients + NPK	259.1b	43.5a	191.7a	7.7 a	94.0 a	5.1 a
10% AE + 25% Micronutrients + NPK	234.7d	35.8b	181.0b	6.9 b	88.7 b	4.7 b

Means sharing the same letters do not differ significantly (P<0.05); data are average of three replicates; AE: acidified extract

Table 2: Effect of acidified extract on different cob related parameters of maize plant

Treatment	Cob length (cm)	Cob diameter (cm)	Stover yield (g/pot)	Lines per cob	Grain per line	Grain per cob
Control (NPK)	10 d	8 b	25d	7 c	19 d	137 d
10% AE + NPK	12 c	10 b	26 d	9 b	20 cd	170 cd
Micronutrients + NPK	13 bc	11 ab	28.11c	9 b	21 c	185 c
10% AE + 100% Micronutrients + NPK	16 a	14 a	35.98 a	11 a	29 a	319 a
10% AE + 75% Micronutrients + NPK	16 a	14 a	35.78 a	11 a	29 a	325 a
10% AE + 50% Micronutrients + NPK	16 a	14 a	35.77 a	12 a	29 a	335 a
10% AE + 25% Micronutrients + NPK	14 b	9 b	32.01 b	10 b	25 b	242 b

Means sharing the same letters do not differ significantly (P<0.05); data are average of three replicates; AE: Acidified extract

Table 3: Effect of acidified extract micronutrients uptake in plant shoot, root and grains

Treatments	Zinc contents in plants			Iron contents in plants			Manganese contents in plants		
	Shoot (mg/g)	Root (mg/g)	Grain (mg/g)	Shoot (mg/g)	Root (mg/g)	Grain (mg/g)	Shoot (mg/g)	Root (mg/g)	Grain (mg/g)
Control (NPK)	55.66d	71.67c	42.67d	109.48b	95.33d	35.50d	30.05d	51d	32.67d
10% AE + NPK	72.66bc	73.67c	47.67d	110.50b	101.00cd	41.00d	52.14c	71.81c	37.67c
Micronutrients + NPK	70.66c	73.67c	49.00cd	111.33b	102.33bc	44.50c	55.75c	75.04c	42.33c
10% AE + 100% Micronutrients + NPK	82.33a	83.67a	63.33a	115.00a	109.67a	76.50a	86.27a	151.15a	75.00a
10% AE + 75% Micronutrients + NPK	82.67a	82.33a	62.67ab	114.50a	109.65a	76.67a	84.27a	150.81a	74.67a
10% AE + 50% Micronutrients + NPK	80a	79.33ab	61.00ab	114.83a	109.67a	77.17a	84.27a	151.84a	7.67a
10% AE + 25% Micronutrients + NPK	75b	75.33bc	50.33bcd	111.67b	105.00b	70.67b	58.16b	102.29b	50.64b

Means sharing the same letters do not differ significantly (P<0.05); data are average of three replications; AE: Acidified extract

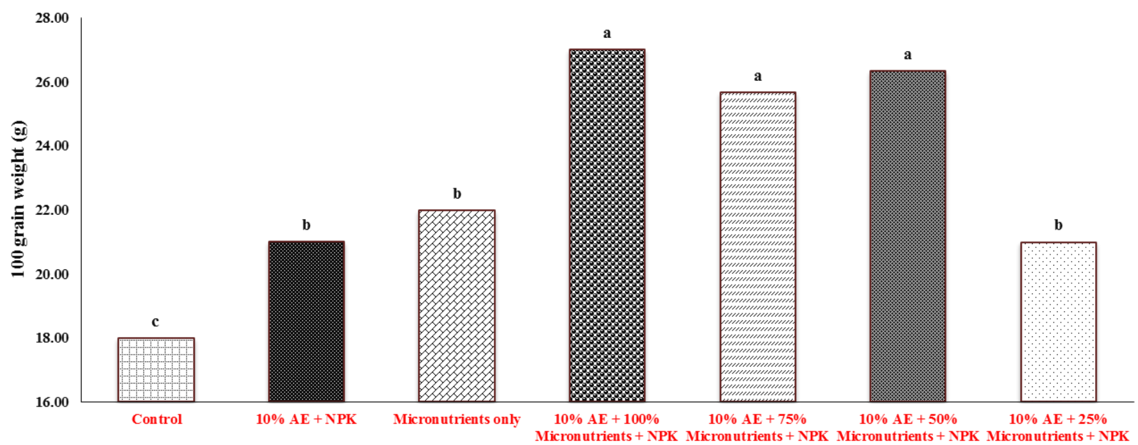

Fig. 1: Effect of acidified extract on 100 grain weight of maize plant; Means sharing the same letters do not differ significantly (P<0.05); data are average of three replicates

and 25% of recommended dose of micronutrient + extract showed maximum increase in Zn uptake in shoot as compared to control and other treatments. These treatments were non-significantly different from each other in Zn uptake pattern. Treatment containing 100, 75, 50 and 25% of recommended dose of micronutrient + extract showed increase up to 21.72, 19.92, 19.01 and 15.39%, respectively of Zn uptake in shoot as compared with control. Similarly, Zn acquisition pattern in root of maize also showed similar trend as was in shoot. Use of 100, 75 and 50% of recommended dose of micronutrient + extract showed 13.5, 11.7 and 7.68% increase compared to separate application of micronutrient while up to 16.74, 14.84 and 10.6% increase, respectively was observed over untreated control. Data regarding Zn uptake in grains also showed the similar trend, that acidified extract along with applied micronutrients considerably enhanced the Zn concentration in grains. All level of applied micronutrients with acidified extract significantly fortified the grain but most noticeable and almost similar outcomes were obtained from 100, 75 and 50% of recommended dose of micronutrients + acidified exact, followed by treatments having 25% of the recommended dose of micronutrients + acidified extract. These three treatments showed 29.24, 27.27 and 24.48% increase in grain Zn compared to separate application of micronutrients.

Iron Concentration in Shoot, Root and Grains of Maize

Data in Table 3 shows the impact of acidified amendment alone and in combination with micronutrients on Fe uptake in maize in comparison with control or other treatments. Although sole application of micronutrients showed enhanced uptake of Fe however, treatments where both micronutrients + acidified extract were applied showed most prominent outcomes regarding Fe uptake due to acidification of extract. Maximum Fe uptake in shoot was detected where recommended dose of micronutrient + extract was applied, however it differed non-significantly from 75 and 50% dose of micronutrients + acidified extract. Treatments (100, 75 and 50% of recommended dose of micronutrients + acidified extract) increased Fe concentration upto 5.50, 5.04 and 5.34%, respectively compared to control. Likewise, same trend was recorded in root Fe contents and in comparison with control, 15.04, 15.02 and 15.04% increase was recorded by combined application of 100, 75 and 50%, respectively along with acidified extract. In case of grain Fe, extract + micronutrients again showed maximum results and in comparison with alone recommended dose of micronutrients, 71.91, 72.25 and 73.41% increase was recorded from 100, 75, 50%, respectively, which was statistically non-significant compared to each other.

Manganese Concentration in Shoot, Root and Grains of Maize

Data regarding Mn concentration in shoot, root and grain is presented in Table 3 which shows the outcomes of micronutrients alone and in combination with extract. Although, treatments with applied micronutrients showed good results but were not as good as treatments with combined application of micronutrients and acidified extract. Treatments with alone application of micronutrients showed significant outcomes but manganese uptake in shoot was maximum where acidified extract was applied with micronutrients, as all the levels of micronutrients showed significant results compared to all other treatments, but, the most prominent outcomes were recorded where micronutrients were applied at the rate of 100, 75 and 50% of the recommended dose with acidified extract @100 liters/acre. If compare these above said treatments with recommended dose of micronutrients, then, 54.74, 51.15 and 51.15% increment and in comparison with control, 187.08, 180.24 and 180. 24% increment was recorded from treatments where micronutrients were applied at the rate of 100, 75 and 50% of the recommended dose with acidified extract @ 247 liters/ha, respectively (Table 3). Similar outcomes were recorded from manganese acquisition in root where maximum uptake was observed from micronutrients + acidified extract containing treatments and when compared these treatments with alone application of micronutrients then, 101.53, 101.08 and 102.45% increment was recorded from 100, 75 and 50% of the recommended dose of micronutrients + acidified extract, respectively. The same trend in Mn concentration in grain was observed in all treatments and in comparison with control, 134.37, 131.25 and 133.25% increment was recorded from 100, 75 and 50% of the recommended dose of micronutrients with acidified extract, respectively. Further, sole application of micronutrients and sole application of acidified extract showed almost same outcomes and, where micronutrients were not applied showed minimum Mn uptake and fortification.

Discussion

Soil pH manipulation through elemental Sº is studied successfully by many researchers (Iqbal *et al.*, 2012; Erika *et al.*, 2012; Ramzani *et al.*, 2016) but main hindrance with its use is that, tones of Sº is required which is not affordable for our farmer's. So, to resolve this issue present study was planned to develop an alternative strategy to enhance indigenous and applied nutrients availability in simplest and beneficial way. For this purpose, an acidified amendment was formulated which lowers the soil pH and make nutrients available for plant uptake.

As, plant's vegetative grow this a clear indicator of any applied inputs and in present work, significant increase in plant growth indicated the positive influence of applied acidified extract which improved the mobilization of micronutrients (Fe, Zn and Mn) in soil through pH manipulation and their uptake to plants. Number of mechanisms might be working behind this growth improvement as, pH alteration by acidified slurry desorbs the nutrient (Pedersen *et al.*, 2017) and causes its mobility into plants (Kimetu *et al.*, 2008), manure slurry acidification can minimize ammonia volatilization (Fangueiro *et al.*, 2015), increase the amount of dissolved nutrients in manure (Christensen *et al.*, 2009) which regulates the plant growth. Further, it might be due to the fact that micronutrients have a pivotal role in plant physiology, enzyme activity (Figueiredo *et al.*, 2012), photosynthesis (Marschner, 1995) and many other physiological processes which significantly improves the plant growth (Liu *et al.*, 2006; Hao *et al.*, 2007; Grzebisz *et al.*, 2008; George *et al.*, 2014). Morphological parameters like shoot, plant height, plant biomass and stem diameter were significantly improved in treatments where micronutrients were applied in combination with acidified extract. Recently, Pedersen *et al.* (2017) reported enhanced nutrient bioavailability and vegetative growth of maize through application of manure slurry acidified with sulfuric acid on sandy soil. Some other workers also advocated the same fact that micronutrients boost up the plant growth attributes including plant height (Lauer, 2006; Soomro *et al.*, 2006; Ziaeyan and Rajaie, 2009), plant biomass (Lewis *et al.*, 2005; Ashrafullah *et al.*, 2010), stem diameter, number of leaves per plant (Baloch *et al.*, 2008; Aslam *et al.*, 2011; Shehzad *et al.*, 2012; Tariq *et al.*, 2014) and other yield related parameters (Marschner, 1995).

In case of grain yield and cob related parameters, combined application of micronutrients + acidified extract showed maximum increase attributed to the higher uptake of nutrients due to lower pH, better root growth and subsequently higher biomass and grain yield. It has been described by Zhao *et al.* (2010) that soil pH was found to play the most important role in determining nutrient solubility from mineral surfaces, minerals speciation, eventual bioavailability of the essential micronutrients and their movement in soil. According to Uprety *et al.* (2009), application of acidified manure can considerably increase the soil micronutrient contents; and in our case manure was provided as a base material which enhanced the micronutrients bioavailability and improved the growth and yield of maize (Bakry *et al.*, 2009; Kruczek, 2005; Lisuma *et al.*, 2006; Singh *et al.*, 2009). Our findings were also in line with Tariq *et al.* (2014); Ghaffari *et al.* (2011); Lana *et al.* (2007) and Baloch *et al.* (2008) who revealed that micronutrients played a critical role in achieving higher yield and growth in maize plant.

Micronutrients uptake in shoot and root of maize was determined and maximum outcomes were recorded from treatments with applied extract and micronutrients both. It might be due to the acid produced by oxidation of Sº which lowered the pH of soil and solubilized the bounded and precipitated nutrients (Malakouti, 2005; Ullah *et al.*, 2014; Havlin *et al.*, 2016). This is further in line with the expectation, that produced H^+ from Sº oxidation replace the metal cations (Zn, Fe, Mn etc.) from organic compounds or mineral surfaces such as iron hydroxide and co-dissolve the other beneficial nutrients from minerals which are then taken up by plants (McLaughlin *et al.*, 1998; Iqbal *et al.*, 2012). Additionally, sulfate produced from Sº oxidation provide negative sites for metal complexation which are easily accessible to plants roots. Additionally, another most interesting hypothesis is that, SOBs which are driving force behind oxidation process may have used the MnOx and Fe(III) (Brock and Gustafson, 1976) as an electron acceptor during oxidation of Sº and resultantly these reduced metals (Fe^{+2}, Mn^{+2}) become available in soil and easily taken up by plant roots (Iqbal *et al.*, 2012). As well, some other researchers experimented on nutrients availability through pH manipulation by application of acids as HNO_3 (Schwertmann *et al.*, 1987), organic acids (citric, galic and oxalic acids) (Renella *et al.*, 2004), acetic acid (Tessier *et al.*, 1979) and found that acids addition mobilize the mineral nutrients through solubilization of carbonates which help to buffer the soils (Schwertmann *et al.*, 1987). Acids helped to dissolve the calcium minerals and this solubility further depends upon several factors such as percentage and type of carbonate present, particle size, and sample size (Kunze, 1965). Acids decrease the buffer power of soil and resultantly nutrients become bio-available, further a clear decrease in distribution coefficient K_{dl} (ratio between C_{sln} and labile metal conc. in solid phase) was also determined which showed that through moderate acidification metal resupply from solid phase can be possible (Muhammad *et al.*, 2012). Resultantly, improvement in micronutrients uptake in plants and characteristics of calcareous soil are accorded (Kayser *et al.*, 2000; Malakouti and Homaei, 2005; Kalich and Golchin, 2008; Siami *et al.*, 2008; Chaghazardi *et al.*, 2014). Concluding, all plant growth, yield and nutrient uptake parameters of maize were increased through combined use of acidified extract and micronutrients compared to control and other treatments. Further, treatments with 50, 75 and 100% of the recommended dose of micronutrients + extract showed same outcomes, ensuring that 50% level is sufficient for plants and no higher input is required as no additional response was recorded by plants at 75 and 100% of micronutrients. This highest response in case of acidified extract was due to facts which solubilize the minerals due to H_2SO_4 and produced of organic acids from microbes which are help full in dissolution and uptake of micronutrients and their accumulation in plant and grains.

Conclusion

Combined application of acidified extract and different levels of micronutrients significantly enhanced all the growth, yield parameters and micronutrients (Zn, Fe and Mn) uptake in plants. Although, alone application of micronutrients showed pronounced outcome compared to control (with no applied micronutrients) but in comparison with integrated use of acidified extract and micronutrients, these results were non-significant. Maximum results were obtained from treatment, where 100% micronutrients + acidified extract was applied however, it differed non-significantly from 50 and 75% of micronutrient + extract. So, on the basis of these outcomes, it is concluded that at 50% micronutrients + acidified extract, plant can take maximum nutrients to attain its sufficiency level and any addition in nutrients cannot give extra benefit to plants. It was due to acidification by virtue of Sº oxidation which lowered the pH of soil for shorter period of time and this pH shock mobilized the soil nutrients and made them highly available for plants. But, this change in pH was only for short time span due to buffering capacity of our soil so, it would be more beneficial to apply this product at peek nutrient requirement of crop. In addition, this product can easily have been prepared and applied at farmer field with minimum cost and to further explore the characteristics to product multi-sites field experiments are needed for enhancing the uptake of macro- and micro-nutrients.

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