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Combined Effects of Elevated Manganese and Zinc Levels on Germination, Chlorophyll Development, Foliar Nitrogen and Carbon Sequestration in *Sorghum bicolor* seedlings

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ABSTRACT: Sorghum is a major staple crop in many parts of semi-arid tropics, but soil nutrient deficiencies and pollution are limiting its performance. The study was carried out to determine the combined effects of elevated levels of manganese and zinc in the soil on the germination and performance of *Sorghum bicolor* seedlings at the early stage of growth. A 9×3 complete block design (CBD) experiment consisting of 10 mg/kg and 100 mg/kg manganese (Mn) levels each with sub-levels of zinc (Zn) at 0 mg/kg, 10 mg/kg, 20 mg/kg and 40 mg/kg were set-up in three replicates alongside the uncontaminated control. *Sorghum bicolor* L. Moench were sown in the treatments after two weeks of spiking. The set-up was observed for germination (as from 1 day after sowing - DAS), cchlorophyll index (at 7 DAS and 14 DAS), leaf nitrogen (at 14 DAS), biomass accumulation of seedlings (at 14 DAS). The results showed that germination occurred in all the treatments at 3 DAS and the combined low (10 mg/kg) Mn with low (10 mg/kg) Zn treatment achieved the highest germination rate (100%) while high (100 mg/kg) Mn without Zn had the least (26.67%). High Mn combined with high Zn improved chlorophyll index and leaf N. Contrarily, increasing levels of Mn had no significant impact on biomass accumulation/carbon sequestration. The study concluded that Mn and Zn spiking differently impacted on the performance indices of sorghum. The combined low levels of Mn and Zn improved germination while increasing levels of both metals enhanced chlorophyll and foliar N concentration in sorghum.

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Sorghum (*Sorghum bicolor* L. Moench) is a major staple crop for the world's poorest and most food insecure people. It is one of the leading drought-tolerant crops that is used to avert the risk of food insecurity and to meet household food needs in many developing countries of the world (Teshome *et al*., 2018). Sorghum is a major source of energy, protein, vitamins and minerals for millions of the poorest people in the semi-arid regions (Stefoska-Needham *et al*., 2015). Despite the immense benefits of the crop and its tolerance to drought, the yield of the crop has been reportedly low, typically less than 30% in Africa compared to those in other parts of the world. This problem has been attributed to nutritional deficiencies associated with the soil nutrients composition (Grundon *et al*., 1987), and the effects pollutants in

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many areas where the plant is cultivated (Godson-Ibeji and Ubochima, 2016). Plants suffer nutrient deficiency stress when availability of soil nutrients, and/or the amount of nutrients taken up, is below that required to sustain metabolic processes in a particular growth stage and the problem is particularly severe if it occurs at the early stage of development. The problem of nutrient deficiencies may result from an inherently low nutrient status of soil, low mobility of nutrients within soil, poor solubility of the given chemical form of the nutrient, or the soil-microbe-plant interactions (Rengel, 2002; Marschner *et al.*, 2011). Soils of most parts of the tropics are often exposed to acidification which lowers the pH, limit major plant nutrients while increasing the availability of other elements e.g. aluminium (Al) and manganese (Mn), which are reportedly toxic to plants at high concentrations (Harter, 2007). Manganese is the eleventh most common element in the earth's crust (Barber, 1995), and forms an essential part of plant metabolism. It aids the development of photosynthetic proteins and enzymes. Its deficit affects the chloroplasts because it limits the photolytic system of photosystem II (PSII), which provides the necessary electrons for photosynthesis (Buchanan *et al.*, 2000). However, Mn is particularly damaging to the photosynthetic apparatus at high concentration (Mukhopadhyay and Sharma, 1991). Thus, it is important to understand the two roles of Mn - both as an essential element at optimum concentration and as a toxic element when in excess (Kochian *et al.*, 2004; Ducic and Polle, 2005). Mn toxicity is favoured in acid soils (Pendias and Pendias, 1992). With decreasing pH, the amount of exchangeable manganese – mainly Mn^{2+} form – increases in the soil solution. This Mn form is available for plants and can be readily transported into the root cells and translocated to the shoots, where it is finally accumulated (Marschner, 1995).

In contrast, other forms of Mn predominate at higher pH values, such as Mn (III) and Mn (IV), which are not available and cannot be accumulated in plants (Rengel, 2000). Excessive Mn concentrations in plant tissues can alter various processes, such as enzyme activity, absorption, translocation and utilization of other mineral elements (Ca, Mg, Fe and P), causing oxidative stress (Ducic and Polle, 2005; Lei *et al.*, 2007). The threshold of Mn injury as well as the tolerance to an excess of this metal is highly dependent on the plant species and cultivars or genotypes within a species (Foy *et al.*, 1988, Horst, 1988). Mn interacts both with cations and anions in oxidation-reduction reactions involving Mn. These reactions are influenced by a variety of physical, chemical and microbiological processes (Bradl, 2004). Zn levels in the soil in many parts of the tropics are reportedly low due to leaching (Sillanpää and Vlek, 1985), and the plant-available fraction is also low because the soil chemistry favours only a sparingly soluble Zn complexes (Rengel, 2002). Zinc, a micronutrient required for plant growth, is influenced by other nutrients in the soil. These nutrients may interact with Zn by affecting its availability from soils and its status in the plant through the processes of growth or Zn absorption, distribution or utilization. In so doing, they may enhance or depress the response of plant growth to Zn. Conversely, Zn may affect other nutrients in a similar manner (Anderson and Thomas, 1946; Anderson, 1956). The present study is aimed at assessing the combined effects of elevated levels of manganese and zinc in soils on the germination and early development of chlorophyll, foliar nitrogen and carbon sequestration in *Sorghum bicolor.*

MATERIALS AND METHODS

Study Area: The study was set up in the Ecology Laboratory at the Department of Plant Biology, University of Ilorin, Ilorin, Kwara state, Nigeria. The University is located on latitude 8.4912° N and longitude 4.5950° E within Ilorin, Kwara state of Nigeria. Ilorin city occupies an area of about 468 km^2 and the climate of area is entirely tropical with the mean monthly temperature of between 33˚C and 34˚C while relative humidity of the area is about 75-80% during the rainy season and near 65% during the dry season (Oyedeji *et al*., 2021).

Soil and Germplasm Collection: The soil for the experiment was loamy top soil collected from fallow vegetation in Ilorin that is relatively uncontaminated with heavy metals. The top soil was collected from different points and bulked together to obtain a homogenous composite soil. The soil was passed through 2 mm mesh sieve and 2 kg were filled into each plastic pot that were used for the cultivation of sorghum. Grains of *Sorghum bicolor* were obtained from Kwara State Ministry of Agriculture and Natural Resources, Ilorin.

Experimental Design and Set-Up: The experiment was set up in a 9×3 complete block design (CBD). The soil treatments consist of low manganese (10 mg/kg) and high manganese (100 mg/kg) contamination levels with each consisting of sub-levels of zinc of 0 mg/kg, 10 mg/kg, 20 mg/kg and 40 mg/kg along with the uncontaminated control soil (without Mn or Zn contamination). Each treatment was replicated three times. Manganese was applied as $MnSO_4.H_2O$ while zinc was applied as $ZnSO_4.5H_2O$. A total of 27 pots were set up. The low Mn (10 mg/kg) and high Mn (100 mg/kg) were applied as 62.16 mg and 621.6 mg of

$MnSO_4.H_2O$ respectively. The zinc treatments of 0, 10, 20 and 40 mg/kg were applied as 0, 79.84, 159.68 and 319.36 mg of $ZnSO_4.5H_2O$ respectively. The soils were moistened with distilled water after manganese and zinc additions and left for two weeks before sowing the grains of sorghum. Five grains of sorghum were sown into each pot.

Evaluation of Variables and Data Collection: The pots were observed for sprouted seedlings from 3 days after sowing (DAS) until 7 DAS. Chlorophyll content in the seedlings was determined at 7 DAS and 14 DAS using SPAD leaf chlorophyll meter. At 14 DAS, nitrogen content in fresh leaves of the seedling was also determined using leaf N meter. The plants were then uprooted to determine the biomass accumulated at 14 DAS using electronic precision balance.

Statistical Analysis of Data: Data collected were subjected to one-way analysis of variance (ANOVA) using SPSS ver. 23 for Windows. Significant means were separated using Duncan's Multiple Range test at $P<0.05$.

RESULTS AND DISCUSSION

The result of seedling germination of *Sorghum bicolor* as influenced by manganese and zinc levels in the soil is presented in Table 1. Seedling emergence in all the treatments started 3 days after sowings (3 DAS). Generally, manganese and zinc levels did not significantly affect ($P > 0.05$) sprouting of the sorghum grains. At 3 DAS, the 10 mg/kg Mn combined with 10 mg/kg Zn treatment achieved 100% germination and was the highest while 100 mg/kg Mn without Zn (0 mg/kg Zn) had the least number of sprouted grains (26.67%). The trend observed at 3 WAS and 4 WAS were similar, except that the number of sprouted grains in the 100 mg/kg Mn without Zn (0 mg/kg Zn) had increased to 40.00%. There was no significant difference in the percentage of sprouted grains/emergent seedlings at 5 DAS, 6 DAS and 7 DAS. The range of percentage germination was 60 – 100% from 5 – 7 WAS (Table 1). It was evident from the results that spiking of soil with low concentration of Mn and Zn together increased the rate of sprouting of sorghum. This was the case in the 10 mg/kg Mn combined with 10 mg/kg Zn that achieved 100% sprouting as early as 3 days after sowing. Despite that no significant difference was observed, the high concentration (100 mg/kg) of Mn in the soil caused a reduction in the germination speed and the number of emergent seedling, even at 7 DAS. The result of germination was consistent with the observation of Kuo and Mikkelsen (1981), who also observed that high manganese in the growth medium (nutrient culture) affected the growth of sorghum. It was observed by the authors that high uptake of Mn by the roots of the plant reduced its potential to absorb other valuable plant nutrients. This was also confirmed by Ullah *et al.* (2017) for bread wheat. Teshome *et al.* (2018) also observed that Zn when used as a priming agent in sorghum improves germination speed and efficiency.

The result of chlorophyll index (CI) in *S. bicolor* as influenced by manganese and zinc levels in the soil is presented in Table 2. There was no significant difference ($P = 0.735$) in the CI of *S. bicolor* among the soil treatments with varying levels of Mn and Zn at 7 DAS. At 14 DAS, CI was significantly different ($P < 0.001$) among the treatments. Chlorophyll index was highest in the plants grown in the untreated soil/Control (27.03) and different from the Mn and Zn treatments. The treatments with 10 mg/kg Mn (low manganese) combined with varying levels of Zn, had incremental CI as Zn levels decreases. The order was 0 mg/kg Zn (17.80) ≥ 10 mg/kg Zn (15.10) ≥ 20 mg/kg Zn (10.13) ≥ 40 mg/kg Zn (5.57). The treatments with 100 mg/kg Mn (high manganese) combined with varying levels of Zn had CI increased with increase in Zn levels, except for the 40 mg/kg Zn which was lower than 20 mg/kg Zn. The order was 0 mg/kg Zn (4.30) ≤ 20 mg/kg Zn (7.27) ≤ 20 mg/kg Zn (11.87) ≥ 40 mg/kg Zn (11.03) (Table 2). Pigmentation, mainly chlorophyll in the sorghum seedlings was unaffected by Mn and Zn levels in the soil at the first week after sowing (7 DAS). The impact of the spiking of the soil with the metals began to manifest at 14 DAS. Low Mn in the soil induced higher chlorophyll in the plant as Zn levels in the soil decreased. Also, high Mn increased the pigment levels as Zn levels increased. This result confirms earlier report that Zn application would enhance growth including pigmentation in cereals, including sorghum (Zulfiqar *et al*., 2020). High concentrations of the nutrient elements, may however, induce toxic effects being a micronutrient.

The result of leaf nitrogen in (mg/g in fresh leaf weight) *S. bicolor* seedlings as influenced by the levels of Mn and Zn in the soil is presented in Table 3. There was significant difference ($P < 0.001$) in the concentration of leaf N. The plants in the untreated/Control soil had the highest leaf N (11.00 mg/g FW) while plants in the 100 mg/kg Mn without (0 mg/kg Zn) had the least concentration of leaf N (3.73 mg/kg). Leaf N decreased with increasing Zn in the low (10 mg/kg) Mn treatments with the order 0 mg/kg Zn (8.23 mg/kg N) ≥ 10 mg/kg Zn (7.43 mg/g N) ≥ 20 mg/kg Zn (6.10 mg/g N) > 40 mg/kg Zn (4.47 mg/g N). Leaf N increased with soil Zn levels in the high (100 mg/kg) Mn treatments. The order was 0 mg/kg Zn (3.73 mg/g N) ≤ 10 mg/kg Zn (4.90 mg/g N)

≤ 20 mg/kg Zn (6.37 mg/g N) = 40 mg/kg Zn (6.13 mg/g N) (Table 3). The result of leaf nitrogen in sorghum aligned closely with that of leaf chlorophyll index. The element decreased significantly with higher Zn levels in the low (10 mg/kg) Mn treatments. The converse was the case in the high (100 mg/kg) Mn treatments, as higher Zn induced higher concentration of foliar nitrogen. Leaf nitrogen correlates with chlorophyll concentrations in the leaf. Zn fertilizer has been shown to increase chlorophyll and net photosynthetic rate in leaves of plants, however, its effectiveness has been shown to be species-dependent (Mao *et al*., 2014; Liu *et al*., 2016).

The result of fresh biomass of *S. bicolor* seedlings as influenced by the levels of Mn and Zn in the soil is presented in Table 4. There was no significant difference (P = 0.648) in the fresh biomass of sorghum in the Mn and Zn-treated soils. The range of biomass at 14 DAS was 206.66 – 258.81 mg FW. The trend of biomass was incremental with Zn levels in the low (10 mg/kg) Mn treatments. In the high (100 mg/kg) Mn treatments, biomass also increased along with Zn levels, except for 10 mg/kg Zn that induced exceptionally high biomass in the category (Table 4). The levels of Mn in the soil had no significant impact on the biomass accumulation in the crop. However, increases along the Zn gradient in the soil were noticed. Exceptional increases in biomass of the crop was observed with high (100 mg/kg) Mn mixed with 10 mg/kg Zn. The `combination may prove to be optimum for increasing growth and carbon assimilation in sorghum. Liu *et al.* (2020) similarly reported that soil application of Zn fertilizers improved shoot biomass as well as grain yield in maize.

Table 1: Germinated seedling of *Sorghum bicolor* grown in manganese- and zinc-contaminated soils at days after sowing (DAS)

Treatment	3 DAS	4 DAS	5 DAS	6 DAS	7 DAS
100 mg/kg Mn + 40 mg/kg Zn	86.67^{a}	86.67^{abc}	93.33^{a}	93.33^{a}	93.33^{a}
100 mg/kg Mn + 20 mg/kg Zn	46.67^{ab}	46.67$_{bc}$	60.00^{a}	60.00^{a}	60.00^{a}
100 mg/kg Mn + 10 mg/kg Zn	80.00^{ab}	80.00^{abc}	80.00^{a}	80.00^{a}	80.00^{a}
100 mg/kg Mn + 0 mg/kg Zn	26.67^{b}	40.00^{c}	60.00^{a}	60.00^{a}	60.00^{a}
10 mg/kg Mn + 40 mg/kg Zn	66.67^{ab}	66.67^{abc}	66.67^{a}	66.67^{a}	66.67^{a}
10 mg/kg Mn + 20 mg/kg Zn	73.33^{ab}	73.33^{abc}	73.33^{a}	73.33^{a}	73.33^{a}
10 mg/kg Mn + 10 mg/kg Zn	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}
10 mg/kg Mn + 0 mg/kg Zn	46.67^{ab}	53.33^{abc}	60.00^{a}	60.00^{a}	60.00^{a}
Control	86.67^{a}	93.33^{ab}	93.33^{a}	100.00^{a}	100.00^{a}
P-value	0.094	0.128	0.216	0.147	0.147

Means with the same superscripted letter(s) are not significantly different (P>0.05).

Table 2: Chlorophyll index of *Sorghum bicolor* seedling grown in manganese- and zinc-contaminated soils at 7 and 14 days after sowing (DAS)

Treatment	7 DAS	14 DAS
100 mg/kg Mn + 40 mg/kg Zn	22.90±0.99^{a}	11.03±1.62^{cde}
100 mg/kg Mn + 20 mg/kg Zn	21.73±4.91^{a}	11.87±0.98^{cd}
100 mg/kg Mn + 10 mg/kg Zn	23.30±5.04^{a}	7.27±2.45^{def}
100 mg/kg Mn + 0 mg/kg Zn	21.87±0.32^{a}	4.30±0.35^{f}
10 mg/kg Mn + 40 mg/kg Zn	24.20±0.61^{a}	5.57±2.47^{ef}
10 mg/kg Mn + 20 mg/kg Zn	23.13±3.76^{a}	10.13±7.01^{cde}
10 mg/kg Mn + 10 mg/kg Zn	25.80±4.07^{a}	15.10±2.60^{bc}
10 mg/kg Mn + 0 mg/kg Zn	23.23±0.91^{a}	17.80±1.74^{b}
Control	26.53±5.42^{a}	27.03±2.11^{a}
P-value	0.735	<0.001

Means with the same superscripted letter(s) are not significantly different (P>0.05).

Table 3: Leaf nitrogen (mg/g FW) of *Sorghum bicolor* seedling grown in manganese- and zinc-contaminated soils at 7 and 14 days after sowing (DAS)

Treatment	Leaf N
100 mg/kg Mn + 40 mg/kg Zn	6.13±0.49^{cd}
100 mg/kg Mn + 20 mg/kg Zn	6.37±0.29^{cd}
100 mg/kg Mn + 10 mg/kg Zn	4.90±0.80^{de}
100 mg/kg Mn + 0 mg/kg Zn	3.73±0.47^{e}
10 mg/kg Mn + 40 mg/kg Zn	4.47±0.72^{e}
10 mg/kg Mn + 20 mg/kg Zn	6.10±1.99^{cd}
10 mg/kg Mn + 10 mg/kg Zn	7.43±0.84^{bc}
10 mg/kg Mn + 0 mg/kg Zn	8.23±0.55^{b}
Control	11.00±0.60^{a}
P-value	<0.001

Means with the same superscripted letter(s) are not significantly different (P>0.05).

Table 4: Fresh biomass (mg) of *Sorghum bicolor* seedling grown in manganese- and zinc-contaminated soils at 14 days after sowing (DAS)

Treatment	Fresh Weight (mg)
100 mg/kg Mn + 40 mg/kg Zn	228.24±44.76^{a}
100 mg/kg Mn + 20 mg/kg Zn	218.41±8.43^{a}
100 mg/kg Mn + 10 mg/kg Zn	241.85±27.20^{a}
100 mg/kg Mn + 0 mg/kg Zn	207.47±12.83^{a}
10 mg/kg Mn + 40 mg/kg Zn	258.81±61.89^{a}
10 mg/kg Mn + 20 mg/kg Zn	258.16±52.44^{a}
10 mg/kg Mn + 10 mg/kg Zn	244.56±21.46^{a}
10 mg/kg Mn + 0 mg/kg Zn	206.66±47.93^{a}
Control	234.09±39.95^{a}
P-value	0.648

Means with the same superscripted letter(s) are not significantly different (P>0.05).

*Conclusion***:** The study concluded that spiking of the soil with manganese and zinc differently impacted on the performance of *Sorghum bicolor* seedlings. The germination of the grains was improved by combined low levels of manganese and zinc. Increasing levels of both metals jointly improved chlorophyll and foliar nitrogen concentration in sorghum seedlings, but carbon sequestration was not significantly impacted by the different levels of both metals combined.

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