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Evaluation of Growth Parameters of *Sorghum bicolor* (L. Moench) in Rusty Soil Treated with Sulfosalicylic Acid

***Ologidi, C.G., Ogbulu, A.O., Sigmus, V., Osia, A., Michael, R., Ebiakpo, D., Apreala, A., Dubia, P.M., Izoukumoh, J.N., Akpoezughe, P.S., Ogheneuyoyo, N.O., Ikpong, C.M., & Igburu, E.**
Department of Biological Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria

***Corresponding author email:** charles.ologidi@ndu.edu.ng

Abstract
Rusty soil is a mixture of rusty sand (a waste from treatment of groundwater), soil, and manure and this mixture has been shown to support optimal plant growth when there is 75%, 12.5%, and 12.5% rusty sand, soil, and manure, respectively. In a bid to improve the growth performance of plants in the rusty soil, which contains high concentration of iron due to precipitation of the micronutrient as ferric ion during groundwater treatment, sulfosalicylic acid was applied as an iron chelator. The experimentation was done under natural conditions of environment by using 5000g each of loamy soil and rusty sand as negative and positive controls, respectively. The sulfosalicylic acid treatments for the experimentation included 10g, 30g, and 50g dissolution of the acid in distilled water and their application on 5000g of rusty sand and rusty soil (75% rusty sand, 12.5% loamy soil, and 12.5% manure). Three-week old sorghum plants were placed in the experimental soils in triplicate and the growth parameters including length of shoot, leaf, width, and root, and mass of the plant parts were monitored. Statistical analysis of the mean values of the parameters at probability value benchmarked at 5% showed that growth performance of the plants in the rusty soil was similar to the performance noticed in the loamy soil. In addition, planting in the rusty soil resulted in better fresh stem biomass and fresh total biomass than planting in the loamy soil and there were significant improvements of shoot length, increase in shoot length, leaf length, increase in leaf length, increase in leaf width and fresh root biomass due to addition of 10g sulfosalicylic acid in the rusty soil. Therefore, the quality of the rusty soil for plant growth was improved by treatment with the iron-chelating sulfosalicylic acid.

Keywords: Rusty Soil, Sulfosalicylic Acid, Iron Chelation, Plant Growth, Rusty Soil

Introduction

Rusty soil is a mixture consisting of 75% rusty sand, 12.5% soil, and 12.5% manure and has been experimentally proven to optimally support plant growth (Ologidi et al., 2024a). The rusty sand of the soil mixture is produced from filtration of groundwater with sand, whose physical and chemical properties are altered due to deposition of iron precipitate. The rusty sand is normally substituted with new sand after too much deposition of iron precipitate in order to ease the removal of the easily oxidizable ferrous ion in the groundwater. Therefore, the rusty sand is a waste and it is usually dumped without thoughtfulness of environmental aftermaths like soil and water contamination, which could be noticed as an unwanted change in physical and chemical properties of a contaminated environment. The impact of mismanagement of the waste could have enormous environmental impact because slow sand filtration of groundwater, due to the simplicity and low-cost implications, is being used by all household having water facility and all commercial water production facilities in Nigeria and other developing countries. But the silver lining is that dumping of the waste on land could serendipitously result in the desired mixture of 75%, 12.5%, and 12.5% rusty sand, soil, and manure, respectively, for plant growth (Ologidi et al., 2024a). In addition, the waste is usable in construction. Furthermore, the usefulness of the waste has been expanded to include agricultural biofortification of sorghum leaf, stem, and root with iron, which is plausible in other crops (Ologidi et al., 2024b). Agricultural biofortification involving soil amendments like organic and inorganic fertilizer applications have been experimentally shown to improve the nutritional value of crops (Lagoriya et al., 2023). The application of specific chemicals for chelation of macro/micronutrients in soil for easier uptake by crops has also been demonstrated (Bloem et al., 2017). Therefore, better usability of the

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rusty soil was explored by application of the iron-chelating sulfosalicylic acid (US05/809,586, 1977). Nevertheless, the ability of sulfosalicylic acid to improve the rusty soil by supporting better plant growth was examined in this study as a first line charge to unboxing more applicability of the waste sand in agriculture.

Materials and methods

Plant material

The plant material used in this study was sorghum seeds (*Sorghum bicolor* L. Moench) that were produced by plants that grew from seeds obtained from a market in Amassoma community in Bayelsa State of Nigeria. The materials were nurtured in loamy soil for three weeks under natural climate and the seedlings were transferred into the prepared soils for experimentation.

Treatment and control soils

The soils used in the experiment were loamy soil, rusty sand, and rusty soil. Loamy soil of 5000g served as negative control and the same mass of rusty sand served as positive control. The rusty soil consisted of 75% rusty sand (which was obtained from the water production facility in the Niger Delta University), 12.5% loamy soil, and 12.5% manure (chicken dung and pieces of wood used in rearing chicken in the Niger Delta University research farm) with a total mass of 5000g. 10g, 30g, and 50g of sulfosalicylic acid (SSA) was dissolved in 4000mL of distilled water and 666 mL from the 4000mL was poured and mixed in the rusty sand and rusty soil making a total of seven treatments and two controls. The parts of the treatments and controls are shown in Table 1.

Table 1: Features of soil treatments

S/N	Rusty sand (%)	Constituents of soil	Mixture of soil (%)	Number of parts in mixture	Proportion of mixture	Mass of mixture of soil (g)	Sulfosalicylic acid treatment (g)
1.	0	Loamy soil (LS)	100	1	LS	5000	0
2.	100	Rusty sand (RS)	100	1	RS	5000	0
3.	100	Rusty sand (RS)	100	1	RS	5000	10
4.	100	Rusty sand (RS)	100	1	RS	5000	30
5.	100	Rusty sand (RS)	100	1	RS	5000	50
6.	75	Rusty sand, loamy soil, manure (RS+LS+M)	75/12.5/12.5	3	6RS/LS/M	3750,625,625	0
7.	75	Rusty sand, loamy soil, manure (RS+LS+M)	75/12.5/12.5	3	6RS/LS/M	3750,625,625	10
8.	75	Rusty sand, loamy soil, manure (RS+LS+M)	75/12.5/12.5	3	6RS/LS/M	3750,625,625	30
9.	75	Rusty sand, loamy soil, manure (RS+LS+M)	75/12.5/12.5	3	6RS/LS/M	3750,625,625	50

LS: loamy soil, RS: rusty sand, M: manure

Site and design for experimentation and transplanting

The experimentation was done in the Department of Biological Sciences in the Niger Delta University with triplicating of each unit of experiment in an entirely randomised fashion. The three-week old seedlings were kept in each unit of experimental soil bucketed in a two-litred container in natural climatic condition.

Data Collection

The parameters that were collected at week of planting (WoT) included length of shoot, leaf, width, and root. These parameters were collected again at 11 weeks after transplant (WAT). In addition, mass of plant parts (leaf, stem, roots, and seeds) was also collected at 11WAT. The fresh total biomass was obtained by summing the mass of the plant parts using formula 1. Furthermore, proportion of fresh root and fresh shoot biomass (it was computed by adding leaf biomass to stem biomass by application of formula 2) was obtained by division of the fresh root biomass with fresh shoot biomass using formula 3. The increments in length of shoot, leaf, and width were obtained by applying formula 4, formula 5, and formula 6, respectively.

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$$\text{Fresh total biomass} = \text{leaf biomass} + \text{stem biomass} + \text{root biomass} + \text{seed biomass} \ldots\ldots.1$$

$$\text{Shoot biomass} = \text{leaf biomass} + \text{stem biomass} \ldots\ldots..2$$

$$\text{Fresh root to shoot ratio} = \frac{\text{fresh root biomass}}{\text{fresh shoot biomass}} \ldots\ldots.3$$

$$\text{Percent increase in shoot length} = \frac{\text{shoot length at 11 WAT} - \text{shoot length at WoT}}{\text{shoot length at WoT}} \text{ x } 100 \ldots\ldots.4$$

$$\text{Percent increase in leaf length} = \frac{\text{leaf length at 11 WAT} - \text{leaf length at WoT}}{\text{leaf length at WoT}} \; x \; 100 \ldots\ldots.5$$

$$\text{Percent increase in leaf width} = \frac{\text{leaf width at 11 WAT} - \text{leaf width at WoT}}{\text{leaf length at WoT}} \text{ x } 100 \ldots\ldots.6$$

Data Analysis and Presentation

Level of variability of mean of the parameters was statistically assessed at probability value benchmarked at 5% by executing the aov and turkeyHSD functions for analysis of variance and separation of mean, respectively, in R (edition 4.1.2) and R Studio on PC (R Core Team, 2021; RStudio Team, 2021). The values were rendered in bar charts by executing the ggplot2 function (Wickham, 2016) with the mean_se and errorbar functions.

Results

There was significant difference between week of planting (WoT) and 11 weeks after transplanting (11WAT) for shoot length of sorghum in the loamy soil and the sulfosalicylic acid-treated rusty soil (Figure 1). But a nonsignificant difference was observed between WoT and 11WAT of the shoot length of the crop in the sulfosalicylic acid-untreated rusty sand and sulfosalicylic acid-treated rusty sand. Furthermore, there was no significant difference of the shoot length at 11WAT for the crop in the loamy soil and sulfosalicylic acid-treated rusty soil of 30g and 50g. The sulfosalicylic acid-treated rusty soil of 10g was however significantly higher than the positive control (that is, shoot length of sorghum in loamy soil) and sulfosalicylic acid-treated rusty soil of 30g and 50g. This significantly higher shoot length that was achieved with the 10g sulfosalicylic acid rusty soil treatment resulted in 3736% increase in the shoot length from WoT to 11WAT and this increase in shoot length was similar to the increase in the shoot length that was obtained with the 50g sulfosalicylic acid rusty soil treatment (Figure 2).

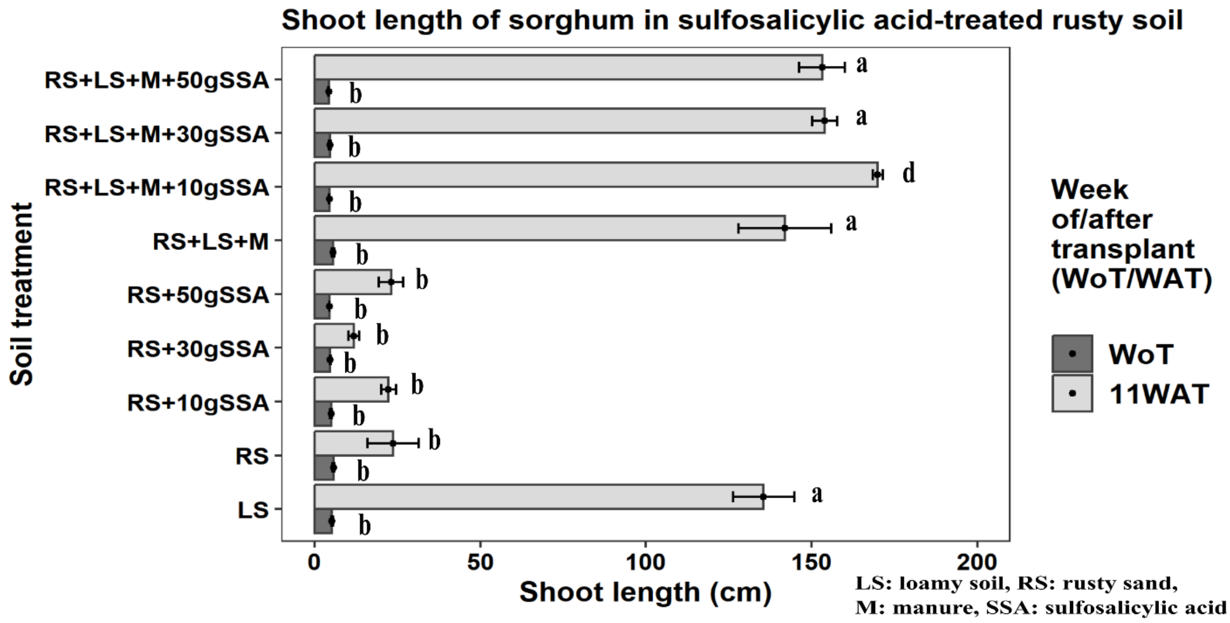

Figure 1: Shoot length of sorghum in sulfosalicylic-acid treated rusty soil

The different letters adjacent to the bars connote statistically meaningful difference in mean and alike alphabets adjacent to the bars express absence of statistically meaningful difference in mean at probability level benchmarked at 5%. Furthermore, alike and unalike numbers express absence of statistically meaningful difference and presence of statistically meaningful difference in average, severally.

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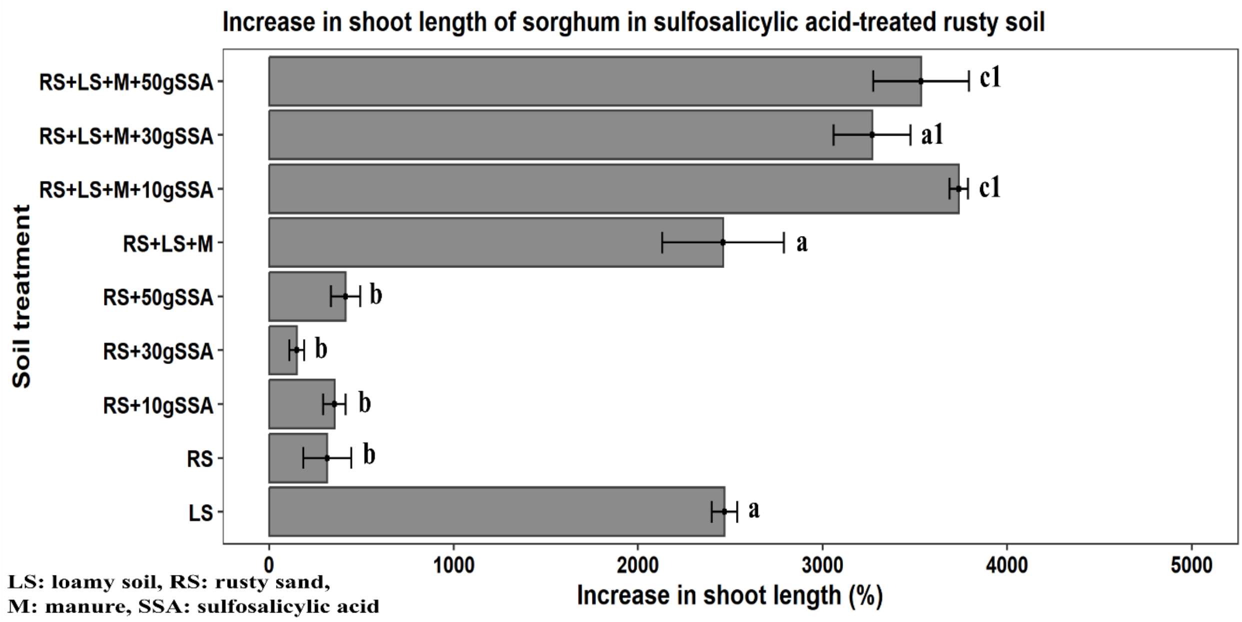

Figure 2: Increase in shoot length of sorghum in sulfosalicylic-acid treated rusty soil

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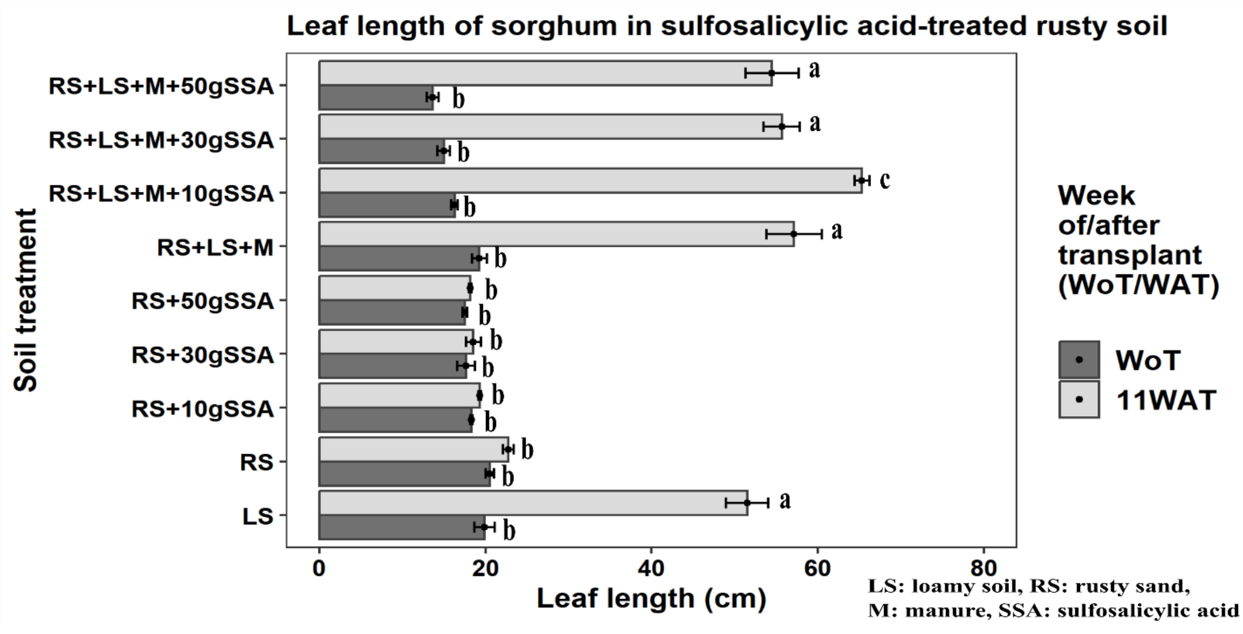

Figure 3: Leaf length of sorghum in sulfosalicylic-acid treated rusty soil

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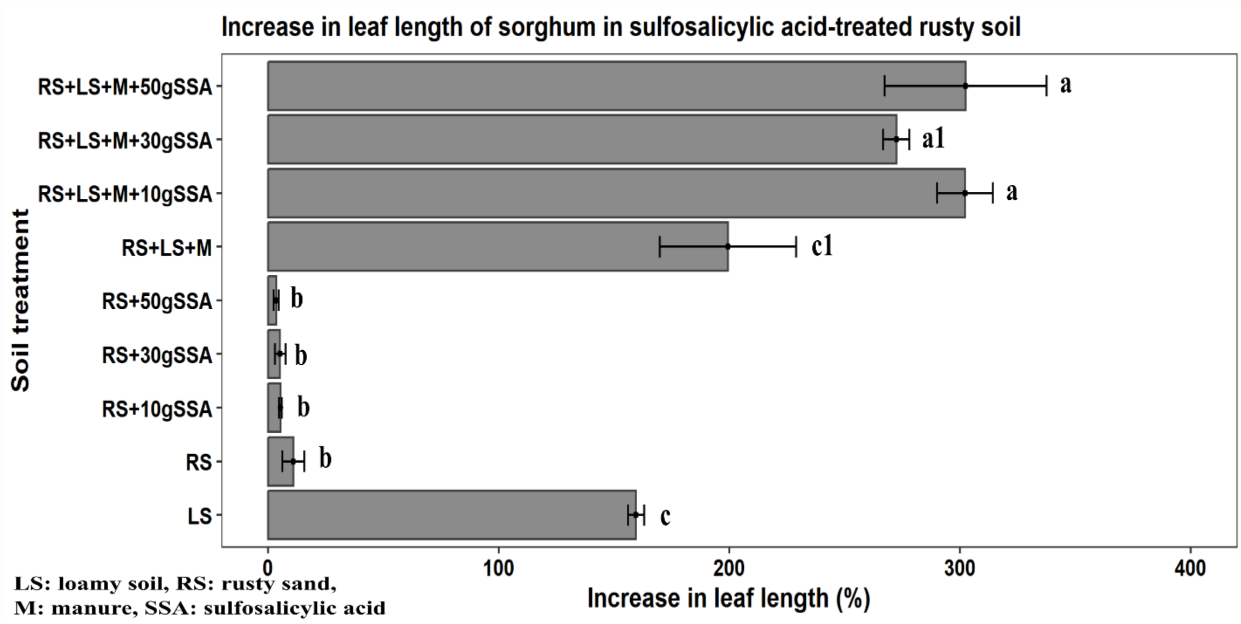

Figure 4: Increase in leaf length of sorghum in sulfosalicylic-acid treated rusty soil

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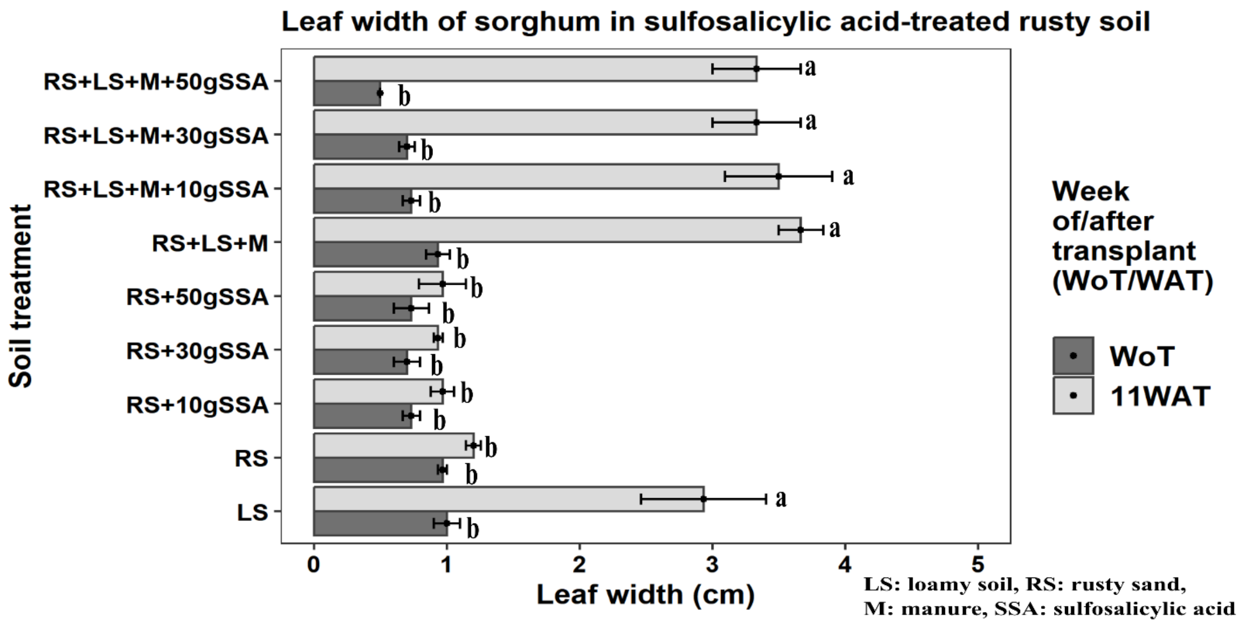

Figure 5: Leaf width of sorghum in sulfosalicylic-acid treated rusty soil

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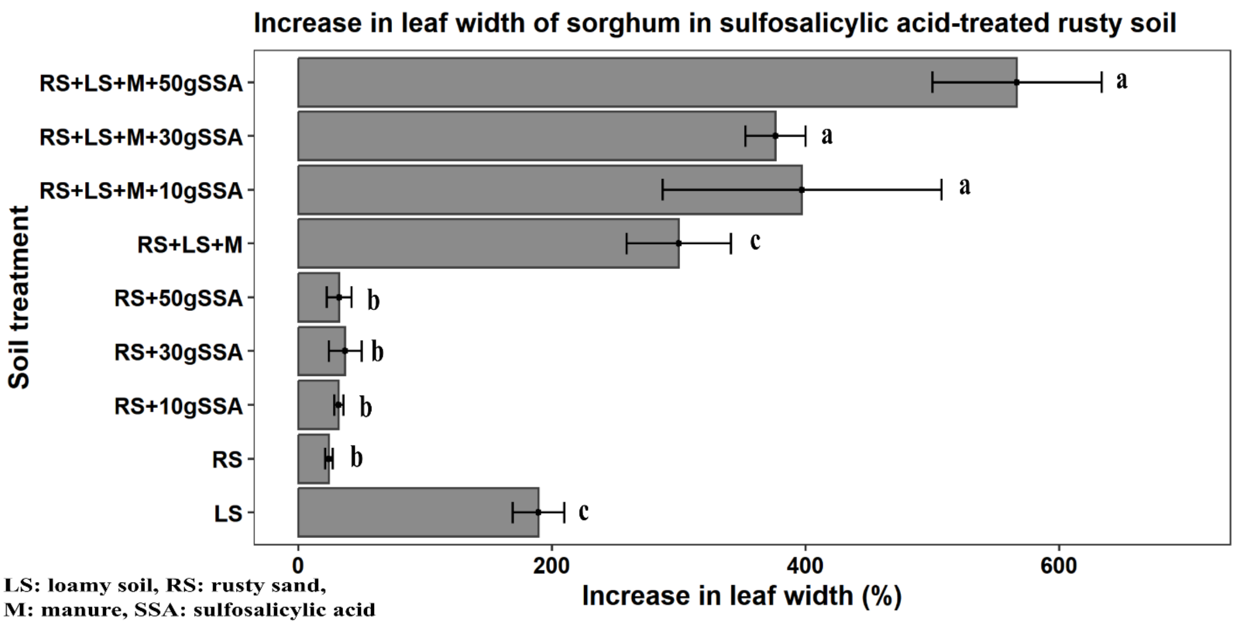

Figure 6: Increase in leaf width of sorghum in sulfosalicylic-acid treated rusty soil

The different letters adjacent to the bars connote statistically meaningful difference in mean and alike alphabets adjacent to the bars express absence of statistically meaningful difference in mean at probability level benchmarked at 5%. The root length was similar in all treatments as there was no significant difference between the treatments and controls (Figure 7). The fresh leaf biomass was also similar in the sulfosalicylic acid-free loamy and rusty soils and the sulfosalicylic acid-containing rusty soils, which were significantly different from the sulfosalicylic acid-free rusty sand and sulfosalicylic acid-containing rusty sand (Figure 8).

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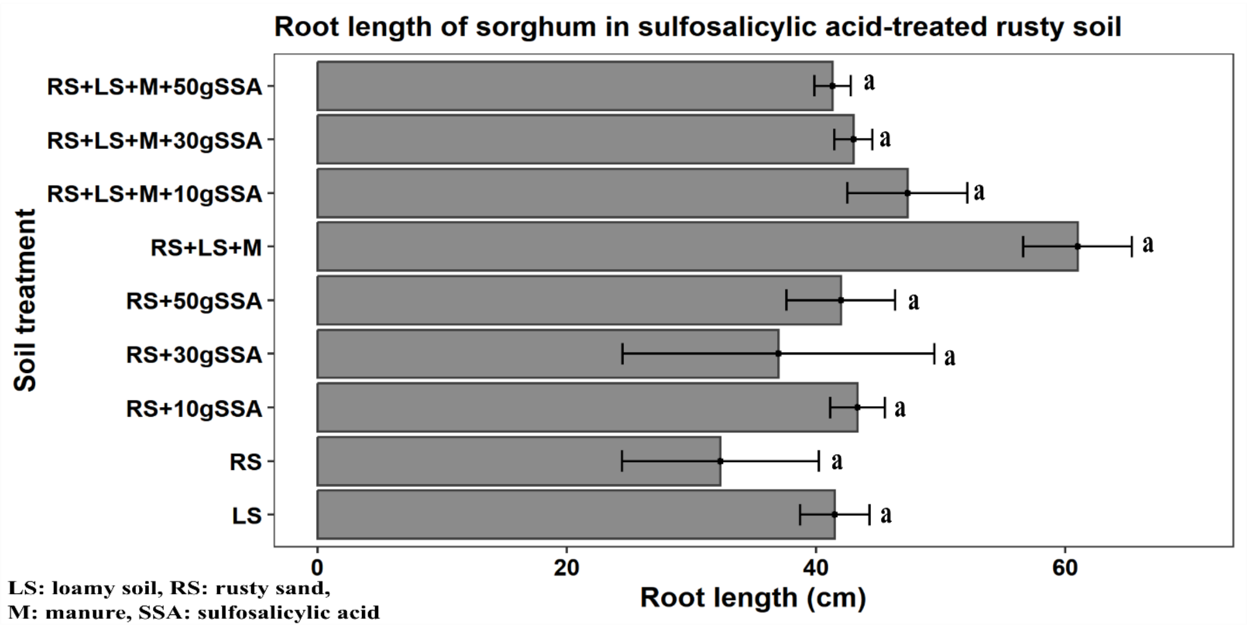

Figure 7: Root length of sorghum in sulfosalicylic-acid treated rusty soil

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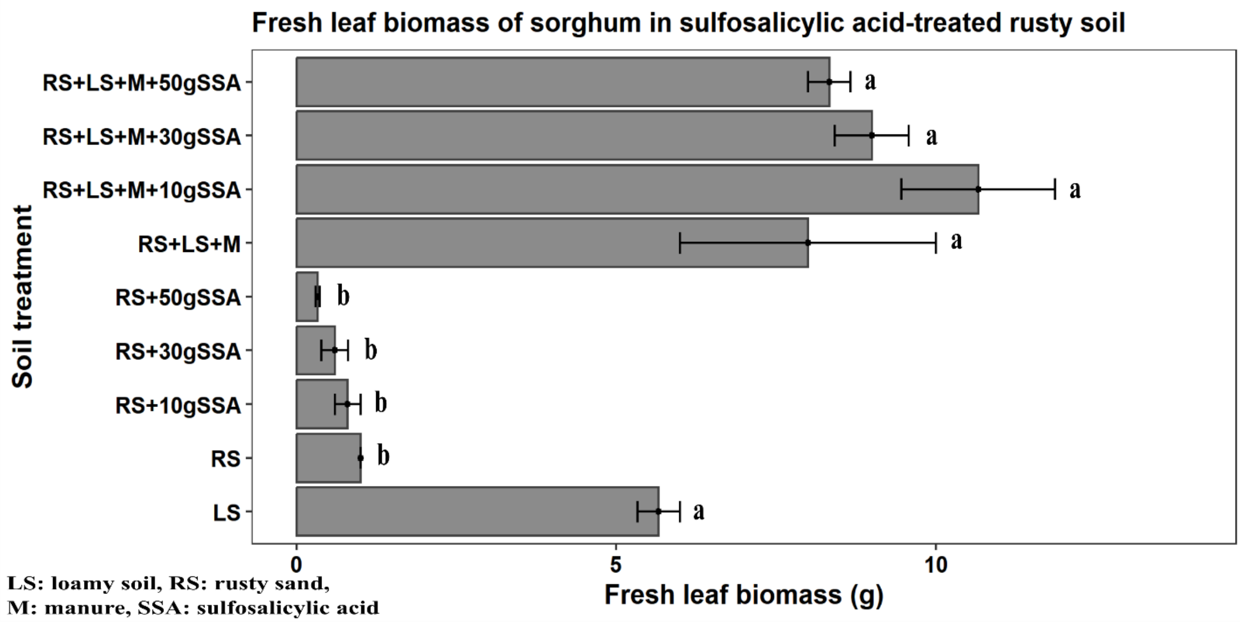

Figure 8: Fresh leaf biomass of sorghum in sulfosalicylic-acid treated rusty soil

The different letters adjacent to the bars connote statistically meaningful difference in mean and alike alphabets adjacent to the bars express absence of statistically meaningful difference in mean at probability level benchmarked at 5%. The fresh stem biomass, fresh root biomass and fresh total biomass produced by the loamy soil were significantly different from the biomass of the rusty sand (both sulfosalicylic acid-treated and untreated) (Figure 9, Figure 10 and Figure 12). But the fresh stem biomass from the loamy soil was similar to the biomass of the 30g and 50g sulfosalicylic acid-treated soils (Figure 9), which were significantly lower than the 10g sulfosalicylic acid-treated rusty soil with a statistical lack of difference between the 10g sulfosalicylic acid treatment and no sulfosalicylic acid treatment in the rusty soil (Figure 9).

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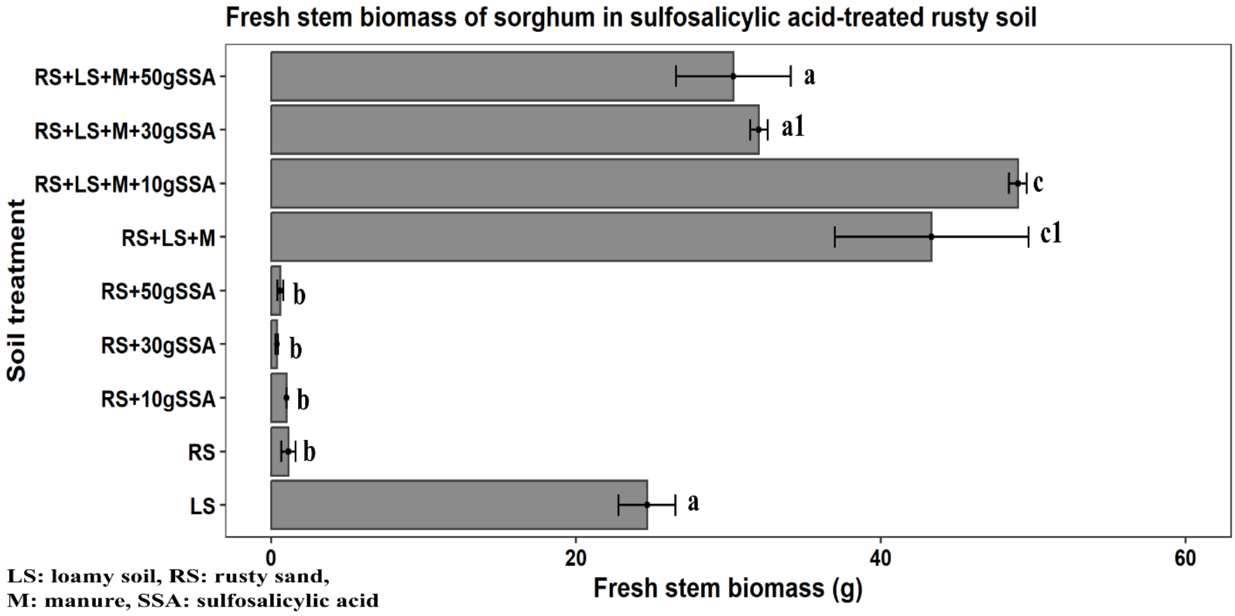

Figure 9: Fresh stem biomass of sorghum in sulfosalicylic-acid treated rusty soil

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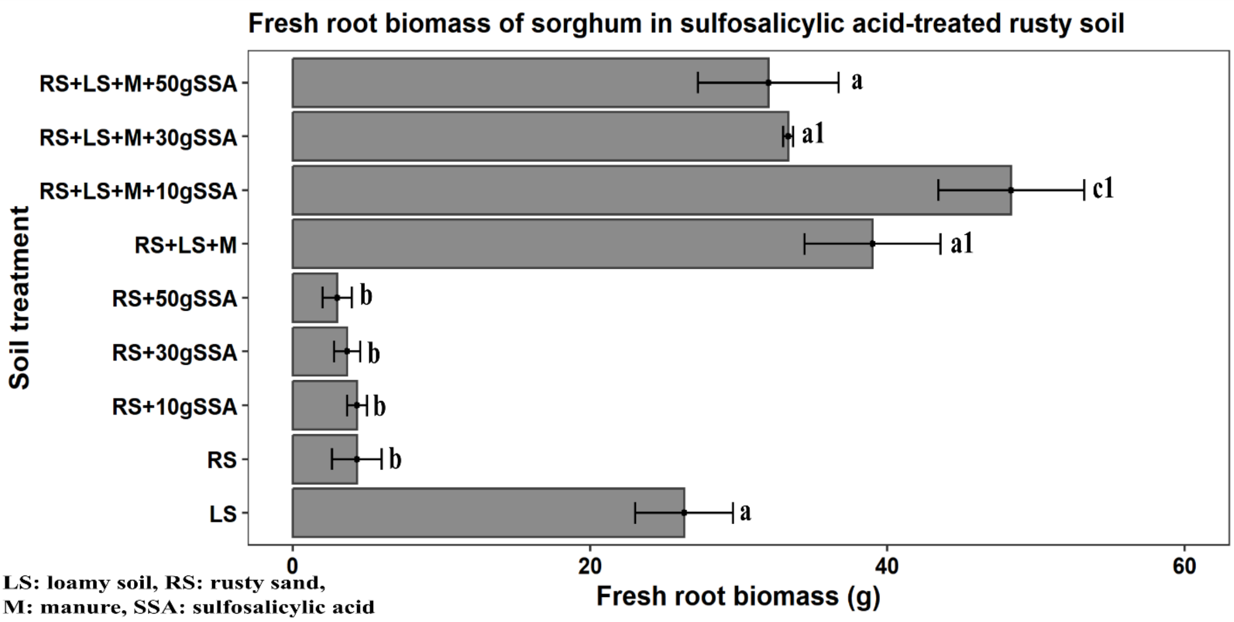

Figure 10: Fresh root biomass of sorghum in sulfosalicylic-acid treated rusty soil

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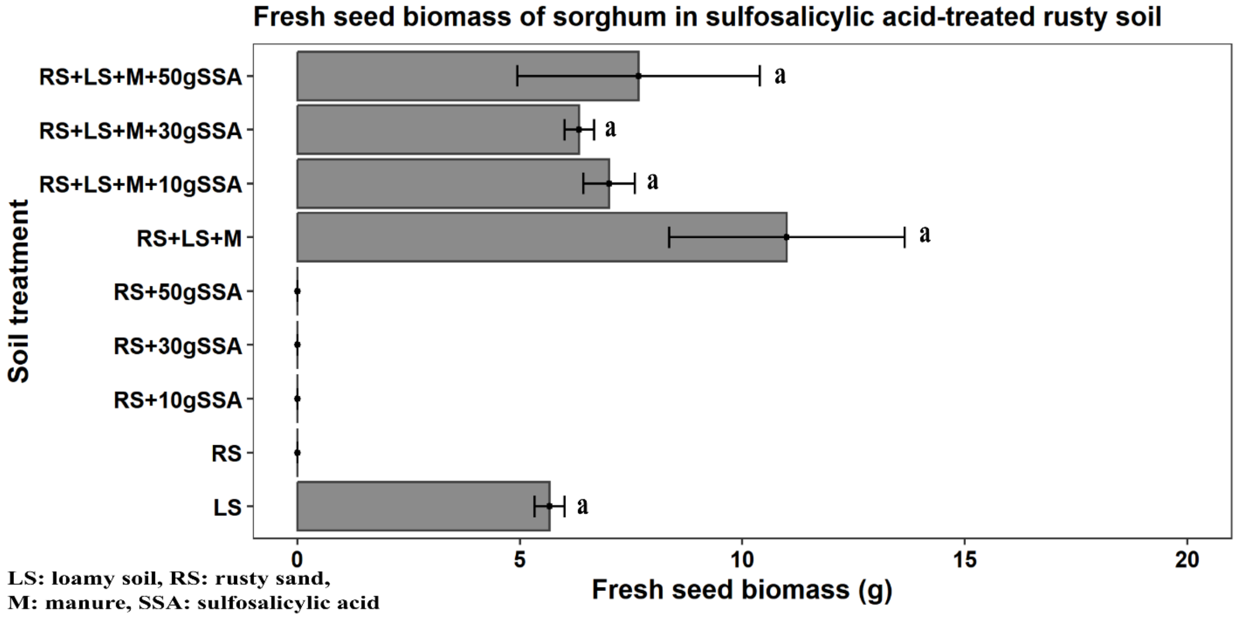

Figure 11: Fresh seed biomass of sorghum in sulfosalicylic-acid treated rusty soil

The different letters adjacent to the bars connote statistically meaningful difference in mean and alike alphabets adjacent to the bars express absence of statistically meaningful difference in mean at probability level benchmarked at 5%. There was significant difference in the fresh total biomass of the plants in the loamy soil and the rusty sand (both sulfosalicylic acid untreated and treated) (Figure 12). There was also significant difference in fresh total biomass between the loamy soil (negative control) and the sulfosalicylic acid-untreated rusty soil and 10g sulfosalicylic acid-treated rusty soils. But the rusty soil with no sulfosalicylic acid treatment showed no significant difference with the 30g and 50g sulfosalicylic acid-treated rusty soils. In addition, the 10g sulfosalicylic acid treatment of the rusty soil resulted in higher significant difference in fresh total biomass of the 10g acid treatment and the two other acid treatments (that is, the 30g and 50g sulfosalicylic acid treatments). The result for ratio of fresh root to shoot biomass of the plants in Figure 13 demonstrates that there was no significant difference between the treatments and controls.

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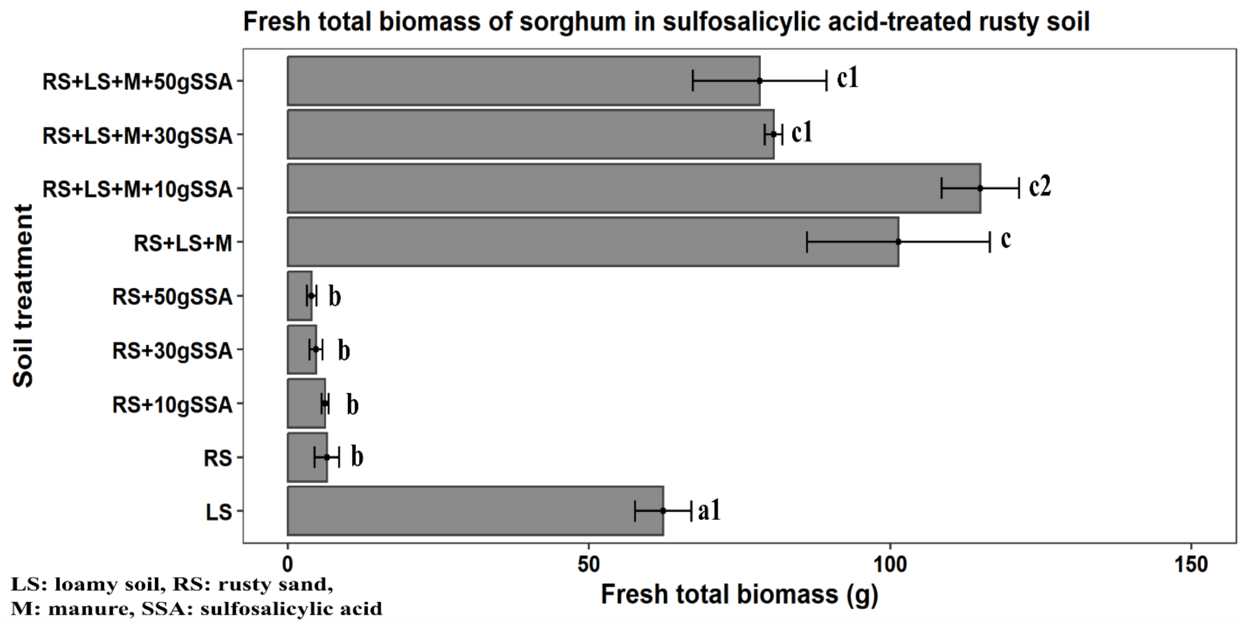

Figure 12: Fresh total biomass of sorghum in sulfosalicylic-acid treated rusty soil

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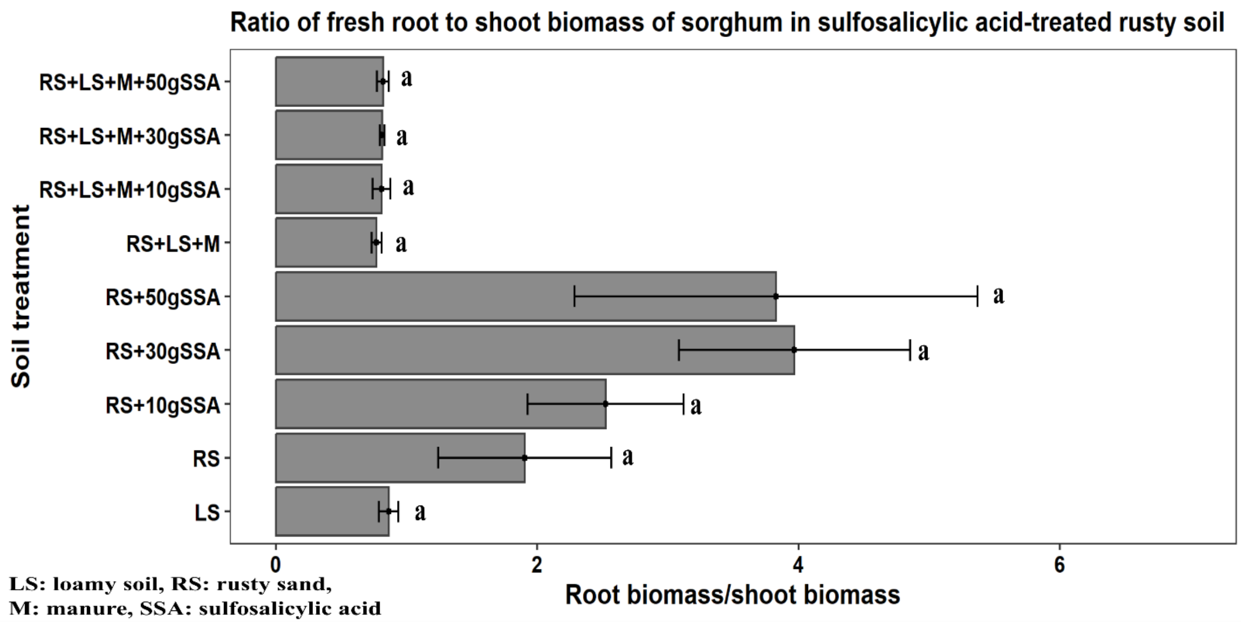

Figure 13: Ratio of fresh root biomass to fresh shoot biomass of sorghum in sulfosalicylic-acid treated rusty soil

The different letters adjacent to the bars connote statistically meaningful difference in mean and alike alphabets adjacent to the bars express absence of statistically meaningful difference in mean at probability level benchmarked at 5%.

Discussion

The observation of the lack of significant difference in shoot length, increase in shoot length, leaf length, increase in leaf length, leaf width, increase in leaf width, root length, fresh leaf biomass, fresh root biomass, fresh seed

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biomass, and ratio of fresh root biomass to fresh shoot biomass for plants in the sulfosalicylic acid-free loamy soil (negative control) and the sulfosalicylic acid-free rusty soil is an indication that the sulfosalicylic acid-free rusty soil supported plant growth like loamy soil. In addition, the noticing of significantly higher fresh stem biomass, fresh stem biomass and fresh total biomass of the plants in the 10g sulfosalicylic acid-treated rusty soil than the sulfosalicylic acid-free rusty soil means that the application of 10g sulfosalicylic acid, which is an iron chelator (US05/809,586, 1977), improved the ability of the rusty soil to support better plant growth. The rusty soil consisting of 75% rusty sand, 12.5% sand, and 12.5% manure produced an optimum growth in plant in a previous study (Ologidi et al., 2024a). But the substitution of the 12.5% sand with 12.5% loamy soil in this study led to a better growth performance such that the 12.5% loamy soil-containing rusty soil produced growth performance that was similar to the loamy soil. Sulfosalicylic acid ($C_7H_6O_6S$), which is otherwise known as 2-hydroxy-5-sulfobenzoic acid, 5-sulfosalicylic acid, and 5-sulphosalicylic acid, is an arenesulfonic acid with a metabolite role (PubChem, 2024). The ability of the acid to chelate cations has been well documented (Xie et al., 2015; Turner & Anderson, 1949) and it forms complex with iron (III) ion (Agren, 1954). Moreover, it is ecofriendly, stable, and atoxic (Xie et al., 2015) thereby making its use as an iron chelator in soil safer than the eco-unfriendly, toxic, and nonbiodegradable ethylenediaminetetraacetic acid (EDTA), which also has proven qualities of cation chelation in soil with plant growth enhancement (Kaurin et al., 2020; Gil-Ortiz & Bautista-Carrascosa, 2007). Thus, the observation of significantly higher fresh stem biomass and fresh total biomass of the plants in the 10g sulfosalicylic acid-treated rusty soil than the sulfosalicylic acid-free rusty soil makes the application of sulfosalicylic acid in enhancing plant growth safer than EDTA. Moreover, salicylic acid, from which sulfosalicylic acid is derived, enhances plant growth and tolerance in biotic and abiotic stress conditions (Al-Taey & Al-Musawi, 2022; Zulfiqar et al., 2021; Bano & Ummat-ul-Habib, 2020).

Conclusion

The rusty soil consisting of 75% rusty sand, 12.5% loamy soil, and 12.5% manure was as good as the loamy soil in supporting plant growth because the rusty soil produced plant growth performance in shoot length, increase in shoot length, leaf length, increase in leaf length, leaf width, increase in leaf width, root length, fresh leaf biomass, fresh root biomass, fresh seed biomass, and ratio of fresh root biomass to fresh shoot biomass that were statistically similar to the growth performance obtained with the loamy soil. Therefore, the rusty soil can be used for growing plants and this could be especially handy in places where agrarian loamy soil may be scarce. As a matter of fact, planting in the rusty soil resulted in better fresh stem biomass and fresh total biomass than planting in the loamy soil. Furthermore, the treatment of the rusty soil with 10g sulfosalicylic acid yielded better results than the results from the loamy soil and the sulfosalicylic acid free-rusty soil as it enabled statistically significant higher growth in shoot length, increase in shoot length, leaf length, increase in leaf length, increase in leaf width and fresh root biomass. Thus, application of 10g sulfosalicylic acid on the rusty soil improved its ability to support plant growth.

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