Investigations on the Bio-Stimulating Potential of Henna (Lawsonia inermis) on Growth and Oil Yield of Lemongrass (Cymbopogon citratus)

Hind M. Ibrahim¹* and Tagelsir I. M. Idris²
¹Department of Horticulture, Ministry of Agriculture and Natural Resources, Sudan
²Department of Horticulture, Sudan University of Science and Technology, Sudan
*corresponding author e-mail: hindm992@gmail.com
Received: 10.02.2020 | Revised: 16.03.2020 | Accepted: 20.03.2020

ABSTRACT
This study aimed to investigate the bio-stimulating potential of Henna (Lawsonia inermis) powder and water extracts on the growth and oil yield of Lemon-grass (Cymbopogon citratus) under nursery conditions at Khartoum, Sudan. Henna powder was tested as soil dressing for Lemongrass in doses of: 0, 4, 8, 12 and 16 g/ plant, while tap water extracts of Henna powder were tested as foliar application on Lemon grass in concentrations of: 0, 4, 8, 12 and 16 g/l. The study was arranged in a complete randomized design where each treatment was replicated six times. Data were collected 6 months after treatments. Considerable gains in growth parameters were obtained upon soil dressing with 16g of Henna powder, whereas these parameters were enhanced substantially by the 8 g/l foliar treatment. Further confirmatory tests are needed coupled with phyto-chemical studies to define the active constituents responsible for these enhancements.

Keywords: Lemongrass (Cymbopogon citratus), Henna (Lawsonia inermis), Growth, Oil content

INTRODUCTION
Lemongrass (Cymbopogon citratus) is a tall herb belonging to the family Poaceae (Akhila, 2010). It is a tropical perennial plant that yields aromatic oils mainly composed of citral which constitutes about 75% (w/w) of the oil (Huynh et al., 2008; Tajidin et al., 2012). Citral has a citrus aroma and is widely used in the perfume and cosmetics industries as well as pharmaceutical preparations and industrial chemicals synthesis (Mestri, 2006; Prommegger et al., 2005; Rauber et al., 2005). The aqueous extract of Lemon-grass is commonly used as a drink while the whole plant is incorporated into traditional food for its lemon flavour (Figueirinha et al., 2008). However, this plant is among flora well adapted to Sudan's agro-climatic conditions (ElGhazali et al., 2004) where few research reports on its agronomy indicated the possibility of its production at field level (Jbreel, 2016). However, the commercial production of this plant in Sudan should be preceded by intensive research efforts.
A plant bio-stimulant is any substance or microorganism applied to plants with the aim to enhance growth and yield attributes. Plant bio-stimulants have been thought to be biological substances that stimulate processes within plants (Saa-Silva 2013). The review of the relevant literature revealed a wide range of growth stimulating compounds including humic and fulvic acids, protein hydrolysates and other N-containing compounds, seaweed extracts, botanicals, chitosan and other biopolymers, beside beneficial fungi and bacteria (Jardin, 2015). The numerous research reports on bio-stimulants documented their positive impact on plant growth, development and productivity, but the mechanism of their action is poorly or not understood (Brown & Saa, 2015; Bulgari et al., 2015). However, most of the interpretations of these enhancements were based on assumptions such as better nutrients uptake, tolerance to biotic and a-biotic stresses, growth regulator-like effects or reactive oxygen/nitrogen species (Patrick 2015). The hormonal–like effects were proposed as causes of enhancements upon application of bio-stimulants (Idris et al., 2011; Colla et al., 2014). Bio-stimulants are safe both for human beings and for the environment and in particular they are valuable for reducing chemicals in agriculture. Reports on growth and yield stimulations in horticultural crops due to their use are frequent under Sudan's conditions (Idris et al., 2011, 2014; Idris & Modawi, 2016).

Henna (Lawsonia inermis L.) belongs to the family Lythraceae. It is traditionally used to develop a red or black coloring to hands, feet and hair in some occasions such as weddings and religious festivals. It is among flora adapted to growth conditions of Sudan where it had been grown in home gardens as hedges and as ornamental. The cultivation of Henna is practiced in Sudan coupled with processing at a commercial level in the River Nile State. According to phytochemical analysis, the powdered of its leaves contain about 0.5-1.5% lawsone; the chief constituent responsible for the dyeing properties of the plant. Henna plant also contains alkaloids, glycosides, flavonoids, saponin, mannnite, tannic and gallic acids, coumarins, mucilage, and naphoquinone (Ahmed et al., 2000; Chukwu et al., 2011; Khan et al., 1991; Kirkland & Marzin, 2003; Nayak et al., 2007; Rosenberg, 1999; Vardamides et al., 2001). Regarding its bio-stimulating property, Chandrasekaran et al. (2000) mentioned that treatment of soyabean seed with Lawsonia inermis leaf extract at 10%, increased shoot length significantly. Besides, Pathak and Srivastava (2000) stated that treating of sunflower with Lawsonia inermis increased its total phenols content. On the other hand, Singh et al. (2006) reported that the extract of Henna gave significant control of the white fly in tomato compared to the untreated control.

In conformity with global trend to replace the synthetic agro-chemicals by natural products to improve growth and productivity of crops, the aim of this study was to investigate the bio-stimulating potential of Henna powder and water extracts on growth and yield of lemon-grass under the conditions of Khartoum State, Sudan.

MATERIALS AND METHODS
This study was conducted in the nursery of the Horticultural Sector Administration, Federal Ministry of Agriculture, Khartoum, Sudan to determine the impact of Henna soil and foliar applications on the performance of lemon-grass plants. The lemongrass experimental materials were obtained from mature field grown plants. Tillers of uniform size and shape were severed to 10 cm length prior to planting in 30X40 cm black polyethylene bags containing alluvial soil. Four weeks after planting, they were used as test material in two separate experiments. The powder of Henna leaves was tested as soil treatments in rates of 0, 4, 8, 12, and 16 g / lemon-grass transplant. The extracts of dry Henna leaves by tap water were tested as foliar treatments in concentrations of 0, 4, 8, 12, and 16 g/l. The two tests were arranged in completely randomized design with 5 replicates. Six months after applications data were collected for the following parameters: number of leaves, leaf length and width, leaf
Ibrahim and Idris  
*Ind. J. Pure App. Biosci.* (2020) 8(2), 1-7  
ISSN: 2582 – 2845

chlorophyll content, shoot and roots fresh and dry weights and leaves oil content. The leaf chlorophyll content was determined with the aid of (Spad device) and the oil content was determined according to AOAC (2003). Data were subjected to analysis of variance and means were separated at 95% confidence limits according to Duncan's Multiple Range Tests with the aid of MStat C computer program.

RESULTS

A. The soil applications:
All Henna soil applications increased the number of leaves significantly over the control. The highest dose (16g/plant) ranked top (Table1). Likewise, all Henna treatments resulted in significant increase in leaf length compared to the control. This parameter was equally enhanced by the 12 and 16 g/plant treatment (Table 1). Regarding leaf width, the best value was recorded for the 4 g/plant Henna treatment, whiles the other treatments induced significant increase over the control at a statistical equal level (Table1). Leaf chlorophyll content was best increased by the 12g /plant treatment compared to other treatments (Table1).

| Henna treatments (g/plant) | Number of leaves | Leaf length (cm) | Leaf width (cm) | Chlorophyll content |
|----------------------------|------------------|------------------|-----------------|--------------------|
| 0                          | 47.50b           | 52.03a           | 1.450a          | 30.38d             |
| 4                          | 51.75b           | 58.27c           | 1.825a          | 33.60b             |
| 8                          | 53.25b           | 60.50b           | 1.550b          | 30.90d             |
| 12                         | 52.50b           | 62.10ab          | 1.625b          | 36.63a             |
| 16                         | 61.25a           | 62.38a           | 1.625b          | 32.40c             |

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limit according to DMRT.

According to Table (2), the 16 g/plant Henna treatment ranked top for the fresh and dry weights of leaves. The 8 g/plant Henna treatment ranked top for the fresh and dry weights of roots. However, the 8 g/plant treatment ranked second for leaves fresh and dry weights, while the 16 g/plant treatment ranked second for roots fresh and dry weights. The 8 g/plant Henna treatment resulted in significant increase in leaf oil content, while the other Henna treatments were ineffective as promoters of this parameter (Table 2).

| Henna treatments (g/plant) | Leaves fresh weight (g) | Leaves dry weight (g) | Roots fresh weight (g) | Roots dry weight (g) | Leaves oil content (%) |
|----------------------------|-------------------------|-----------------------|------------------------|----------------------|------------------------|
| 0                          | 087.5a                  | 31.50c                | 40.13c                 | 33.00e               | 0.1667e                |
| 4                          | 156.5d                  | 46.13d                | 31.63c                 | 25.25d               | 0.1667e                |
| 8                          | 203.3b                  | 64.38b                | 39.75c                 | 33.25c               | 0.2333c                |
| 12                         | 173.1c                  | 59.00c                | 32.00d                 | 26.88c               | 0.1667e                |
| 16                         | 312.3a                  | 86.88a                | 36.75b                 | 31.63b               | 0.1333c                |

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limit according to DMRT.
B. The foliar applications:
The 8 g/l Henna treatment was the most enhancive for the number of leaves/plant and leaf length. However, all Henna treatments increased the leaf length over the control (Table 3). The widest leaves were obtained from the 4 g/l treatment, while the highest leaf chlorophyll content was recorded for the 4 and 8 g/l treatments (Table 3).

Table 3: Impact of foliar application of Henna on the number, length, width and chlorophyll content of Lemongrass leaves

| Henna extract conc. (g/l) | Number of leaves | Leaf length (cm) | Leaf width (cm) | Chlorophyll content |
|--------------------------|------------------|------------------|-----------------|-------------------|
| 0                        | 47.25c            | 51.78a           | 1.425bc         | 30.38c            |
| 4                        | 46.00c            | 63.63b           | 1.700a          | 33.33a            |
| 8                        | 81.25a            | 65.28a           | 1.525b          | 33.53a            |
| 12                       | 80.00abc          | 52.15d           | 1.425bc         | 30.70bc           |
| 16                       | 79.00b            | 58.22c           | 1.575b          | 32.10abc          |

* Means with the same letter(s) in the same column are not significantly different at 95% confidence limit according to DMRT.

According to (Table 4), all Henna treatments increased leaves fresh and dry weights significantly over the control. The best values were recorded for the 12 g/l Henna treatment. The 16 g/l Henna treatment also ranked top for both roots fresh and dry weights, followed by 8 g/l Henna treatment. The 4 and 12 g/l treatments were also enhancive for these parameters compared to the control. However, the 4, 8 and 12 g/l Henna treatments resulted in slight but insignificant increase in leaf oil content, while the 16 g/l treatment resulted in slight insignificant decrease compared to the control (Table 4).

Table 4: Impact of Henna foliar applications on fresh and dry weights of leaves and roots, and leaf oil content of Lemongrass plants

| Henna extract conc. (g/l) | Leaves fresh weight (g) | Leaves dry weight (g) | Roots fresh weight (g) | Roots dry weight (g) | Leaves oil content (%) |
|--------------------------|-------------------------|-----------------------|------------------------|----------------------|------------------------|
| 0                        | 087.5e                  | 031.5e                | 40.13e                 | 33.00e               | 0.1333e                |
| 4                        | 189.0d                  | 059.3d                | 46.00d                 | 38.13d               | 0.1667d                |
| 8                        | 313.1b                  | 079.8c                | 54.63b                 | 47.13b               | 0.1667d                |
| 12                       | 335.3a                  | 108.8a                | 50.50c                 | 43.63c               | 0.1667a                |
| 16                       | 306.1c                  | 099.9b                | 143.0a                 | 111.0a               | 0.1333a                |

* Means with the same letter(s) in the same column are not significantly different at 95% confidence limit according to DMRT.

DISCUSSION
The chances of Sudan in the agricultural business are numerous; yet this potential is not fully exploited due to lack of a master plan to exploit the huge resources in accordance with standard modern agribusiness. Hence, there is need to focus on environment friendly and sustainable means to increase the productivity per unit area. Organic farming renders better returns than chemically produced crops. In organic production, bio-fertilizer and bio-pesticides are used instead of agri-chemicals to grant provision of safe food without harming the environment. The core and objectives of this study lied within this context as the growth stimulating potential of Henna was tested on Lemongrass; a plant well adapted to Sudan's agro-climatic conditions (ElGhazali et al., 2004). The data analysis of this test revealed substantial growth gains upon Henna application, and this finding might be considered as a first report claiming of the...
growth stimulating property of this plant especially when used as foliar treatment in concentration of 12 g/l; a treatment that increased the leaf fresh weight by 352.3% coupled by oil content slightly increased over the control. Likewise, the soil application of 6 g Henna was also stimulating of leaf weight as it increased this parameter by 202% beside significant increase in leaf oil content. The employment of these cheap doses of Henna proved to be of significant impact on growth of Lemongrass and this potential can be tested on other horticultural crops for further confirmation. Regarding interpretation, this stimulation might owe growth stimulating hormones or their precursors or a bio-pesticide constituent within Henna tissues. Nevertheless, these findings are in line with other preceding studies on the growth stimulation by applications of local flora of Sudan as substitutes for agro-chemicals. Within this context, Idris et al. (2011) reported enhanced growth and yield in date palm by soil application of low doses Argel. Similarly, other researchers reported growth stimulation by soil and foliar applications of local flora on Aloe vera (Eisa, 2016), Catharanthus roseus (Jbreal, 2016), Mangifera indica (Idris and Albashir, 2018), Duranta plumier (Hamid, 2016) and Euphorbia splendenes (Osman, 2017).

However, further research on the growth bio-stimulation potential of Henna is needed. Further phyto-chemical studies to define the phyto-chemicals responsible for the stimulations of growth might provide solid interpretation of the results. Yet, the encouraging high growth rates of Lemongrass obtained in this study might warrant further research on this plant aiming towards its production at an economical level.

REFERENCES
Ahmed, S., Rahman, A., Alam, A., Saleem, M., Athar, M., & Sultana, S. (2000). Evaluation of the efficacy of Lawsonia alba in the alleviation of carbon tetrachloride-induced oxidative stress. J. Ethnopharm., 69, 157-164.

Akhila, A. (2010). Chemistry and Biogenesis of Essential Oil from the Genus Cymbopogon. Essential oil bearing plants. The genus Cymbopogon. CRC Press, Taylor and Francis Group, Boca Raton, Florida, USA.

AOAC, (2003). Association of Official Analytical Chemists. Official Methods of Analysis. 15th edition. Washington.

Brown, P., & Saa, S. (2015). Biostimulants in agriculture. Front. Plant Sci., 6, 671. doi:10.3389/fpls.2015.00671.

Bulgari, R., Cocetta, G., Trivellini, A., Vernieri, P., & Ferrante, A. (2015). Biostimulants and crop responses: A review. Biol. Agric. Hortic., 31, 1–17.

Chandrasekaran, A., Narasimhan, V., & Rajappan, K. (2000). Effect of plant extracts, antagonists and chemicals on seedling vigour and anthracnose disease of soybean. International J. Tropical Plant Diseases, 18, 141-146.

Chukwu, O. O. C., Odu, C. E., Chukwu, D. I., Hafiz, N., Chidozie, V. N., & Onyimba, A. (2011). Application of extracts of Henna (Lawsonia inamis) leaves as a counter stain. African Journal of Microbiology Research, 5(21), 3351-3356.

Du Jardin, P. (2015). Plant biostimulants: definition, concept, main categories and regulation. Sci.Hortic. 196, 3–14. doi:10.1016/j.scienta.2015.09.021

Eisa, E.M. (2016). Impact of nutrients and biostimulants on growth and yield of Aloe vera plants. Ph.D. Thesis (Horticulture), Sudan University of Science and Technology.

ElGhazali, G. E. B., Abdalla, W., ElEgami, A.A. B., AlMogboul, A. Z. I., & Daim Hamad, A.A. (2004). Aromatic Plants of Sudan. Al Shima Printing House, Khartoum, Sudan.

Figueirinha, A., Paranhos, A., Pe’rez-Alonso, J. J., Santos-Buelga, C., & Batista, M.T. (2008). Cymbopogon citratus leaves: Characterisation of flavonoids by HPLC–PDA–ESI/MS/MS and an
approach to their potential as a source of bioactive polyphenols. *Food Chemistry*, 110, 718–728.

Hamed, O.B.A. (2016). Impact of Argel applications on growth of Golden Duranta. M.Sc. Thesis (Horticulture), *Sudan University of Science and Technology*.

Huynh, K.P.H., Maridabe, J., Gaspillo, P., Hasika, M., Malaluuan, R., & Kawasaki, J. (2008). Essential Oil from Lemongrass Extracted by Supercritical Carbon Dioxide and Steam Distillation. *The Philippine Agric. Sci.*, 91, 36–41.

Idris, T.I.M., Albashir, I.I.(2018). Impact of Argel and Haza shoot water Extracts on seed Germination and seedling Growth of ‘Kitchener’ mango cultivar. M. Sc. Thesis, Sudan University of Science and Technology.

Idris, T.I.M., Abdelrahman, E.M., & Mohamed –Ahmed , I.A. (2016b). Response of Aloe plants to foliar and soil applications of argel (*Solenostemma argel* Del., Hayne).

Idris, T.I.M., Ibrahim, A.M., Elnour, H.S., & Mahdi, E.M. (2014). Effect of differrent forms of Argel (*.Solenostemma argel* Del., Hayne) applications on flowering, fruit set and fruit retention in vegetatively malformed ‘Tommy Atkins’ mango cultivar. *SUST J. Agric. and Veter. Sci.*, 15, 79-86.

Idris, T.I.M., Ibrahim, A.M., Mahdi, E.M., & Taha, A.K. (2011). Influence of Argel (*.Solenostemma argel* Del.,Hayne) soil applications on flowering and yield of date palm (L.). *Agric., & Biol. J. of North Amer.*, 2(3), 538-542.

Jbreel, E. A. A. (2016). Effect of Urea Fertilizer on Growth and Oil Content of Lemongrass (*Cymbopogon citratus* DC. Stapf). M. Sc. Thesis, Sudan University of Science and Technology.

Khan, M.M., Ali, A., Jain, D.C., Bhakuni, R.S., Zaim, M., & Thakur, R.S. (1991). Occurrence of some antiviral sterols in *Artemisia annua*. *Plant Sci.*, 75, 161-165.

Kirkland, D., & Marzin, D. (2003). An assessment of the geno-toxicity of 2-hydroxy-1, 4-naphthoquinone, the natural dye ingredient of Henna. *Mutation Res.*, 537, 183-199.

Mestri, S. D. (2006). Important isolates of Essential oils, *Fafai*, 6(1), 53-57.

Nayak, B., Isitor, G., Davis, E., & Pillai, G. (2007). The evidence based wound healing activity of *Lawsonia inermis* Linn. Phytotherapy Re., 21, 827-831.

Osman, A. A. (2017). *Growth and bloom bio-stimulation in Euphorbia splendenes by Argel (Solenostemma argel) use*. M.Sc. Thesis, Sudan University of Science and Technology.

Pathak, D., & Srivustava, M.P. (2000). Effect of fungicides, plant extracts and biocontrol agents on total phenols content of sunflower plants. *Annals of Biol.*, 16, 227-229.

Patrick, D.J. (2015). Plant biostimulants: definition, concept, main categories and regulation. *Scientia Horticulture*. 196, 3-14.

Prommegger, W., Budur, K., Dorji, & Chhetri, P.B. (2005). Lemon grass distillation in Eastern Bhutan- a scenario analysis. RNR-RC Jakar/CORET, SDF and RNR-RC Whengkhar.

Rauber, C. S., Guterre, S., & Schapoval, E. E. S. (2005). LC determination of citral in *Cymbopogon citrates* volatile oil. *J. Pharm. Biochem. Analysis* 37, 597-601.

Rosenberg, N.M. (1999). Antibacterial deodorizing compositions containing extracts of *Lawsonia inermis*. PCT. Int. Applied, 37.

Saa-Silva, S., Brown, P.H., Ponchet, M. (eds). (2013). First World Congress on the Use of Biostimulants in Agriculture.
Singh, S., Choudhary, D.P., & Mathur, Y.S. (2006). Effect of plant extract against whitefly (*Bemisia tabaci* Genn.) on tomato. *Indian J. Entomology, 68*, 71-73.

Tajidin, N.E., Ahmad, S.H., Rosenani, A.B., Azimah, H., & Munirah, M. (2012). Chemical composition and citral contents in lemongrass (*Cymbopogon citratus*) oil at three maturity stages. *Afr. J. Biotechnol., 11*, 2685–2693.

Vardamides, J.C., Dongo, E., Nkengfack, A.E., Fomum, Z.T., Ngando, T.M., Vogler, B., & Kraus, W. (2001). Diterpenoids and Limonoids from the stem of *Pterorhachis zenkeri*. *Fitoterapia, 72*, 386-393.