Journal of Agriculture and Environment
Vol. 20 No. 2, 2024: 243-250

Microbial biocontrol and drought stress management using orange peels on spinach (*Spinacia oleraceae*) in Sokoto, semi-arid environment

G.A. Abubakar and Murtala Usman

Department of Soil Science and Land Resources Management, Faculty of Agriculture, Usmanu Danfodiyo University, Sokoto, Nigeria

ABSTRACT

A screenhouse experiment was conducted to determine the effectiveness of orange peels powder on spinach growth and productivity grown under soil water-deficit spinach condition in Sokoto, Sudan savannah zone of Nigeria. The orange peel was applied at a rate of 50 g to 5 kg of soil per pot. Growth and yield parameters such as plant height, number of leaves, leaf length, leaf area, and fresh weight and microbial population of the soil were determined using standard methods. The experiment was laid out in a randomized complete block design (RCBD) with four irrigation intervals 2, 4, 6 and to 8 days replicated three times and data were subjected to analysis of variance (ANOVA) significant differences among means were separated at 5% level of significance. Results showed that soil amendment with 50g of orange peels per 5 kg of soil and irrigation intervals at 4 days increased spinach yield and suppressed bacterial population higher than the fungal population in the soil. These findings suggest that the frequency of irrigation had a major effect on spinach yield and production cost, controlling watering intervals increase the spinach yield and reduce the production cost. Further research is needed to investigate the influence of orange peels on microbial diversity, optimal dosage of orange peel, and method of application for enhancing spinach yield.

Keywords: Biocontrol; drought; orange peels; semi-arid; spinach

INTRODUCTION

Orange peel is a primary waste fraction in the production of orange juice, which contains flavonoids associated with antioxidant activity (Ortiz-Sanchez *et al.*, 2024). Orange peel is a by-product during the processing of fruit, and they are good sources of bioactive compounds (Saleh *et al.*, 2021). Every year large amount of orange peels is formed during the production of orange juice, and other orange products, which accumulates in bulk that are rich in nutrients and contain many phytochemicals which are useful in many drugs, agriculture and food industries (Manthey *et al*., 2001). Orange peels are also rich source of flavonoids and ascorbic acids, containing more concentration than in the edible part, meaning that several compounds will be retained in the peel (Lachos-Perez, 2018). Vitamin C and ascorbic acid are the most widely distributed secondary metabolites that are involved in the response to stress (Cheynier *et al.*, 2013).

https://www.ajol.info/index.php/jagrenv
ISSN: 1595-465X (Print) 2695-236X (Online)
correspondence: garba.aliyu@udusok.edu.ng
https://dx.doi.org/10.4314/jagrenv.v20i2.21

Spinach (*Spinacia olaraceae* L.) is a leafy green vegetable crop with short growth life cycle that belongs to the family *Amaranthacea* and subfamily *Chenopodiaceae* (Ribera *et al.*, 2020). It contains minerals and vitamins that facilitate digestion (Habimana *et al*., 2014). It is also rich in vitamins and minerals such as vitamin A, vitamin C, vitamin E, vitamin K, vitamin B2, vitamin B6; and Magnesium, Manganese, Folate, Betaine, Iron, Calcium, Potassium, Folic acid, Copper, Phosphorus, Zinc, Niacin, Selenium protein and omega-3 fatty acids (USDA, 2005). It has high nutritive value with good cooking (Nishihara *et al*., 2001).

Drought stress is one of the most important abiotic stress factors that limits spinach production in many regions in arid and semi-arid areas coupled with climate change (Kucharski *et al.*, 2016). Drought stress in plants is an important factor affecting biochemical and physiological functions limiting growth and development (Srivastava and Srivastava, 2014). Drought is one of the main causes of the decline in crop output coupled with other abiotic stresses such heat stress, salt stress (Ashraf, 2008).

Orange peel contains citric acid, flavonoids and phytochemicals making it a powerful antioxidant that are involved in the response to stress, essential oils, which have a variety of biological uses such as antibacterial and antifungal (Espiard, 2002; Cheynier *et al.*, 2013). Furthermore, orange peels contain potassium, which is essential for plant growth (Nossier, 2021). The main objective of this study was to determine the effectiveness of utilizing orange peel as drought stress and microbial biocontrol agents for enhancing spinach growth in the Semi-arid environment.

MATERIALS AND METHODS

The study was conducted at a screenhouse of Crop Science Department, Faculty of Agriculture, Usmanu Danfodiyo University Sokoto, Sokoto State. Located in extreme North-Western part of Nigeria, near the confluence of Rima River, it is situated on latitude 13º 01’N and longitude of 5º 15'E, which fall within the Sudan Savannah Agro-ecological zone of Nigeria. It has an annual average temperature of 30.6°C with maximum daytime temperatures for most of the year generally under 40 °C and annual rainfall amounts ranging from 390 mm to 790 mm (Ekoh, 2020). The experiment was laid out in a Randomized Complete Block Design (RCBD), replicated three times with four irrigation intervals of 2 days without orange peel (control), 4, 6, and 8 days plus 50 g of orange peel powder which was air dried for three days, ground and applied for 4, 6, and 8-days irrigation intervals added to 5 kg of soil. The experimental spinach seed was sourced from the International Institute of Tropical Agriculture (IITA) in Kano, Nigeria. Six (6) seeds for this trial were directly broadcasted and later thinned to three plants per pot to encourage vigorous growth. All agronomic practices were followed based on the recommendations from the IITA.

Nutrient Agar (N.A) powder (5.6 g) was prepared for bacteria, which was suspended in 200 ml of distilled water, mixed and heated with frequent agitation on a hot plate until dissolved. It was then sterilized by autoclaving at 121°C for 15 min and cooled to 45°C. The media was then dispensed into petri-dishes and allowed to solidify for some minutes. Culture media for fungi was prepared in a sterilized material according to M38-A2 protocol. Sabouraud Dextrose Agar (7.8 g) and antibiotic (streptomycin) were suspended in 200 ml of distilled water, heated with frequent agitation on a hot plate and boiled for one minute to completely dissolve the medium, autoclaved at 121°C for 15 min, and cooled to 45-50°C. The media was then dispensed into petri dishes and allowed to solidify for two hours. A series of test tubes was prepared for the isolation of bacteria and fungi. Nine (9) ml of sterile distilled water was put into each of the test tubes, one gram of the soil sample was added to give a dilution of 10^{-1}. Contents were shaken properly, and 1 ml of the solution was added to the next test tube containing 9 ml of distilled water to make a concentration of 10^{-2}. The serial dilution was made up to 10^{-6} dilution for the soil sample. The culture petri dishes were spread with 0.1 ml of the 10^{-6} dilution using the spread plate technique for bacteria while 10^{-3} for fungi using Sabouraud Dextrose Agar (Cheesbrough, 2010). The plates were then incubated upside down at 37 °C for 24 hr and 96 hr for bacteria and fungi, respectively. Individual colonies developed were then counted.

RESULTS AND DISCUSSION

Plant Height

The result on the effectiveness of orange peel on plant height used as super absorbent and anti-stress under water deficit on spinach is presented in Table 1. Irrigation intervals at 2 days performed better than irrigation at 6- and 8-days intervals and was significant at 3 weeks after sowing but statistically similar with 4 days irrigation intervals. This indicates that frequent irrigation at 2 and 4 days promotes spinach growth in terms of height, and the long irrigation intervals can lower the plant height. However, at 4 WAS, as plant height increasing steadily over time shows no significant differences among the treatments which were statistically similar likewise, at 6 WAS. This implied that water-deficit conditions had a negative impact on spinach growth. Moisture stress had negative effects on spinach underground and aboveground (biomass), and the yield (Ors and Suarez, 2017). Water stress caused 50% significant losses in fresh weight aboveground of spinach (Xu and Leskovar, 2015).

Table 1: Plant height at 3, 4, 5 and 6 weeks after sowing (WAS)

Irrigation Intervals	Plant Height (cm/plant)			
	3 WAS	4 WAS	5 WAS	6 WAS
2 days (Control)	6.33^{a}	7.37	10.6^{a}	13.7
4 days	5.20^{ab}	7.03	9.53^{ab}	12.9
6 days	3.57^{b}	6.23	8.40^{ab}	9.80
8 days	4.06^{b}	6.12	6.50^{b}	9.00
P Value	0.04	0.06	0.01	0.16
SE±	0.42	0.40	0.63	0.86
	*	Ns	*	Ns

SE = Standard error, Ns = Not significant, * = Significant. Values in same column with the same letters did not differ significantly at 5% level significance.

Number of Leaves Per Plant

The number of leaves per plant is presented in Table 2. The results showed that at 3 and 4 WAS there was no significant difference among the treatments. Irrigation at 6 and 8 days at 5 WAS were statistically the same, and irrigation at 2 days has the highest mean, followed by 4-days intervals, while at 6 WAS, 2 and 4 days are similar, and 6 days intervals are intermediate between them, and the 8-days intervals the least performance. Likewise at 6

WAS irrigation intervals of 2, 4 and 6 days are statistically similar. This indicates that orange peels have the tendency to be used as anti-stress for soil moisture (Mishra *et al.*, 2022).

Table 2: Number of leaves at 3, 4, 5 and 6 weeks after sowing (WAS)

Irrigation Intervals	Number of Leaves per Plant			
	3 WAS	4 WAS	5 WAS	6 WAS
2 days (Control)	9.00	12.0	18.0[a]	18.0[a]
4 days	10.0	12.0	14.0[ab]	17.0[a]
6 days	9.00	11.0	12.0[b]	12.0[ab]
8 days	7.00	10.0	10.0[b]	10.0[b]
P Value	0.17	0.47	0.02	0.04
SE±	0.51	0.54	1.22	1.42
	Ns	Ns	*	*

SE = Standard error, Ns = Not significant, * = Significant. Values in the same column with the same superscript letters did not differ significantly at 5% level

Leaves area

The results of the leaves area are presented Table 3. Irrigation intervals at 8 days, had the lowest leave area at 6 WAS, and the treatment with 6 days intervals had the highest leaves area at 6 WAS, and similar with a 2 days interval, but at 2 and 4 days were not significant. This indicates that at 6 days interval spinach performed better in the leaves area, an advantage of saving the cost of irrigation and having a higher yield. This result was in agreement with the findings that a leaf water potential decrease in response to water deficit, the leaf osmotic potential also decreases to maintain positive turgor adjustment, enabling spinach to withstand moderate water stress (Jones *et al*., 1981), this could be attributed to orange peel's being a super absorbent and anti-stress agent, which helped to mitigate the negative effects of water deficit on spinach growth. In terms of spinach growth, Khan *et al*. (2019) observed that addition of orange peel to soil increased spinach yield by up to 25% and reduced oxidative stress in spinach plants under drought conditions.

Table 3: Leaves area at 3, 4, 5 and 6 weeks after sowing (WAS)

Irrigation Intervals	Leaves Area (cm^2)			
	3 WAS	4 WAS	5 WAS	6 WAS
2 days (Control)	6.67	9.50	13.2[b]	18.1[a]
4 days	4.87	5.25	6.58[b]	6.65[b]
6 days	5.34	7.11	24.4[a]	25.5[a]
8 days	3.20	4.13	4.87[b]	5.60[b]
P Value	0.683	0.532	0.03	0.02
SE±	0.92	1.27	2.57	2.73
	Ns	Ns	*	*

Values in the same column with the same letters did not differ significantly at 5% level significance. SE = Standard error, Ns = Not significant, * = Significant.

Fresh Weight

The results on the final fresh weight of spinach Figure 1 showed that soil amendment with orange peels can mitigate water stress on spinach. The irrigation interval at 2 days (control), where no orange peels were added, was similar on the final fresh weight ($p \leq 0.05$) with irrigation intervals at 4 and 6 days where the orange peels were added. However, the highest fresh weight (0.27 kg) was recorded in 2-days irrigation interval followed by 4, 6 and 8 days respectively. The treatment with irrigation interval of 8 days had the lowest fresh weight (0.01 kg). The fresh weights of spinach plants decreased by the imposition of drought stress. Similar results were observed by Khaki-Moghadam and Rokhzadi (2015) and Simon-Grao *et al.,* (2016) in tomato, spinach, and sunflower grown under water stress, plants tend to close their stomata, leading to a significant low rate of transpiration and respiration (Diaz-Lopez *et al*., 2012). Drought stress decreased the growth of roots and shoots, partitioned dry matter, and upset plant-water relations and photosynthesis, thus ultimately affected crop fresh yield (Ashraf, 2010).

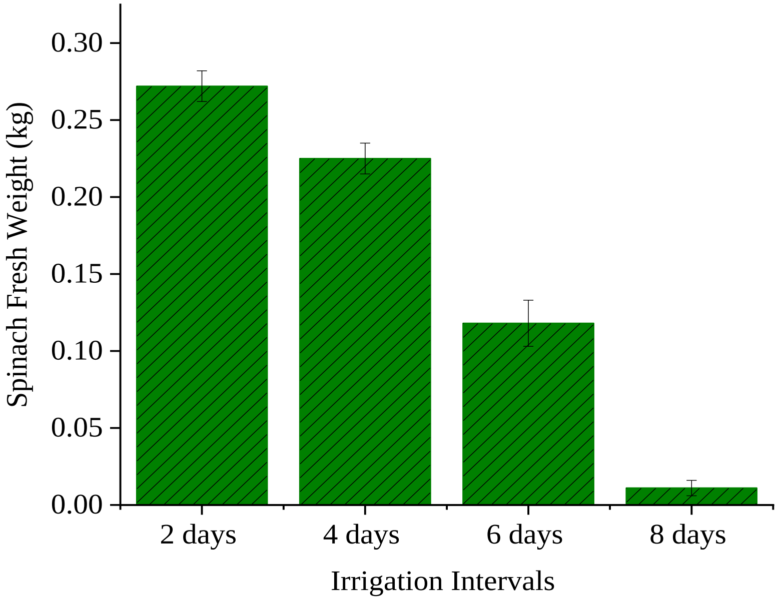

Figure 1: Spinach yield fresh at 2, 4, 6 and 8 days irrigation intervals.

Bacterial and Fungal population

Based on the results shown in Figures 2a, and b. There were no significant differences observed within the bacteria and fungi populations at different irrigation intervals. The highest bacterial count was observed at 2 days irrigation intervals (3.4 x 10^{-6} cfu g^{-1}). Bacterial population were more suppressed than the fungal population within the same treatments. This could be attributed to environmental factors such as moisture, temperature and soil pH. The results showed that the use of orange peel as a water retention agent did not statistically affect the bacterial and fungal populations of the soil. This finding is consistent with previous studies that have shown that the use of agricultural waste as a water retention agent does not significantly affect soil microbial communities (Kumar *et al.,* 2019).

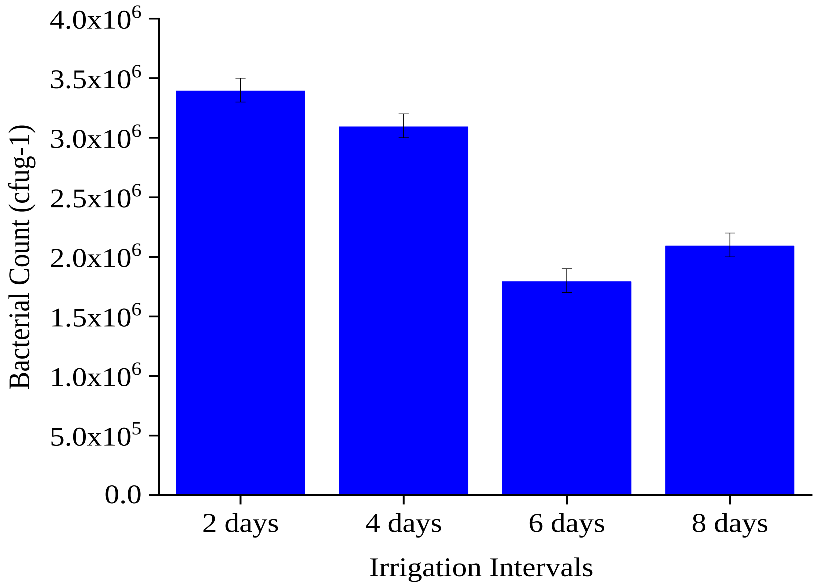

Figure 2a: Bacterial population count at 2, 4, 6 and 8 days irrigation intervals

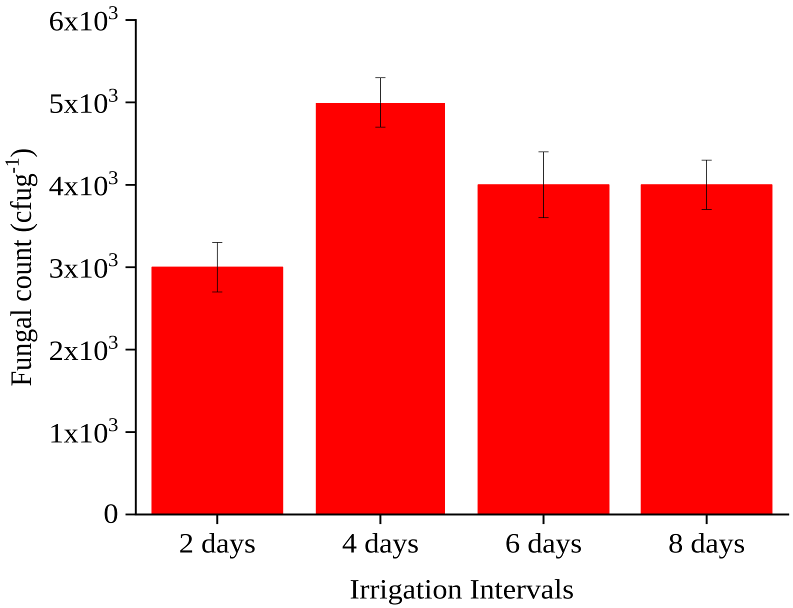

Figure 2b: Fungal population count at 2, 4, 6 and 8 days irrigation intervals

CONCLUSION

It is evident that orange peels can be used for drought management and as biocontrol agent on spinach under semi-arid environment. Also, spinach yield could be improved when soil is amended with orange peels and the irrigation intervals of 4 days could serve as a cost-effective mechanism to lower the cost of production. The results obtained indicated that increasing irrigation intervals from two to four days did not significantly affect spinach yield under water deficit conditions. This was attributed to orange peel being used as a super absorbent and anti-stress agent, which helped to mitigate the negative effects of the water deficit on spinach growth. Further research is needed to investigate the optimal dosage of orange peel, and method of application for enhancing spinach yield.

REFERENCES

Ashraf, M.A. (2008). *Water Use Efficiency in Plant Biology*. Oxford: UK: Blackwell, pp. 1-26.

Ashraf. M. (2010). Inducing drought tolerance in plants: Some recent advances. *Advances in Biotechnology*, 28: 169-183

Cheesbrough, M. (2010). *District Laboratory Practice in Tropical Countries Part II*. 2nd ed. Cambridge University Press, Cambridge, UK; 1-442. 11. Clinical and Laboratory Standards

Cheynier, V., G. Comte, K. M. Davies, V. Lattanzio and S. Martens. (2013). Plant phenolics: Recent advances on their biosynthesis, genetics, and ecophysiology. *Plant Physiology and Biochemistry, 72:1-20.*

Díaz-López, L., Gimeno, V., Simón, I., Martínez, V., Rodríguez Ortega, W.M. and García-Sánchez, F. (2012). *Jatropha curcas* seedlings show a water conservation strategy under drought conditions based on decreasing leaf growth and stomatal conductance. *Agricultural Water Management*, 105: 48-56.

Ekoh, H. C. (2020). Analysis of rainfall trend in Sokoto State, Nigeria (1987-2016). World News of Natural Sciences, 28p.

Espiard, E. (2002). Introduction à la transformation industrielle des fruits (Ed) TEC and DOC, France: 259- 265.

Habimana, S., F. Ndagijimana, F. Uwase and J. R. Migambi. (2014). Production of spinach as influenced by spacing and urea fertilizer in Busogo conditions. *Agri. Sci. Res. J.* 4(12): 223-230.

Jones, M. M., N. C. Turner and C. B. Osmond. (1981). Mechanisms of drought resistance. *In*: L.G. Paleg and D. Aspinall (eds.) *The physiology and Biochemistry of Drought Resistance in Plants*. Academic Press, Sydney, Australia. Pp. 15-37.

Khaki-Moghadam, A. and Rokhzadi, A. (2015). Growth and yield parameters of sunflower (*Carthamus tinctorius*) as influenced by foliar methanol application under well-watered and water deficit conditions. *Environmental and Experimental Biology*, 13: 93-97.

Khan, M., Khan, M., and Khan, M. (2019). Effects of organic waste materials on physiological responses and yield attributes of spinach (Spinacia oleracea L.) under drought stress conditions. *Journal of Agricultural Science and Technology*, 14(3), 466-474. https://doi.org/10.5539/jast.v14n3p466.

Kucharski, J., Sulewski, P., and Rzemieniak, M. (2016). The effect of soil moisture storage capacity on plant performance under drought. *Journal of Water and Land Development*, 31(1): 75-80.

Kumar, S., Singh, R. and Singh, V. (2018). Effect of agricultural waste on soil water retention capacity and crop productivity: A review. *Journal of Environmental Management*, 226: 237-249.

Lachos-Perez, D. (2018). Subcritical water extraction of flavanones from defatted orange peel. *Journal of Supercritical Fluids*, 138,7–16. https://doi.org/10.1016/j.supflu.2018.03.015.

Manthey, J. A., Guthrie, N., Grohmann, K. (2001). Biological properties of citrus flavonoids pertaining to cancer and inflammation, *Current Medicinal Chemistry*, 8, 135–153.

Mishra, R., Singh, S. and Singh, R. (2020). Effect of orange peel as a super absorbent on wheat growth under water stress. *Journal of Applied Research*, 14(2), 178-183.

Nishihara, E., Inoue, M. K., Kondo, Takahashi, K. and N. Nakata. (2001). Spinach yield and nutritional quality affected by controlled soil water matric head. *Agri. Water Manag.*,51(3): 217-229.

Nossier, M. (2021). Impact of organic fertilizers derived from banana and orange peels on tomato plant quality. *Arab Universities Journal of Agricultural Sciences*, 29(1), 459-469.

Ors, S. and Suarez, D.L. (2017). Spinach biomass yield and physiological response to interactive salinity and water stress. *Agricultural Water Management*, 190: 31-41. doi: 10.1016/j.agwat.2017.05.003

Ortiz-Sanchez, M., Cardona Alzate, C.A. and Solarte-Toro, J.C. (2024). Orange peel waste as a source of bioactive compounds and valuable products: insights based on chemical composition and biorefining. *Biomass*, 4(1), 107-131.

Ribera, A., Bai, Y., Wolters, A.M.A., van Treuren, R., and Kik, C. (2020). A review on the genetic resources, domestication and breeding history of spinach (*Spinacia oleracea* L.). *Euphytica*, 216(3), 48.

Saleh, M., Amro, L., Barakat, H., Baker, R., Reyash, A.A., Amro, R., and Qasem, J. (2021). Fruit by-product processing and bioactive compounds. *Journal of Food Quality*, 2021(1), 5513358.

Simon-Grao, S., Garcia-Sanchez, F., Alfosea-Simon, M., Simon, I., Lidon, V. and Ortega, W.M.R. (2016). Study on the foliar application of fitomare on drought tolerance of tomato plants. *International Journal of Plant Animal & Environmental Sciences*, 6: 15-21.

Srivastava, S. and Srivastava, M. (2014). Morphological changes and antioxidant activity of Stevia rebaudiana under water stress. *American Journal of Plant Sciences,* 5 (22): 3417.

USDA, (2005). USDA National nutrient database for standard reference, release 18. Nutrient Data Laboratory. U.S. Department of Agriculture, Agricultural Research Service. http://www.nal.usda.ov/fnic/foodcomp.

Xu, C. and Leskovar, D.I. (2015). Effects of seaweed extracts on spinach growth, physiology and nutrition value under drought stress. *Scientia Horticulturae*, 183: 39-47.doi: 10.1016/j.scienta.2014.12.00468.