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EFFECTS OF SOIL SOLARIZATION ON FUNGAL AND BACTERIAL POPULATIONS ASSOCIATED WITH AMARANTHIS VIRIDIS L. (AFRICAN SPINACH) IN LAGOS, NIGERIA

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ABSTRACT

Soil solarization is increasingly used to control soil-borne pathogens because it is environment-friendly. However, performance varied geographically, necessitating experimental trials before its introduction. This study assessed the effects of solarization on soil-borne bacteria and fungi, growth, and proximate composition of *Amaranthus viridis* (African spinach) in Lagos, Nigeria. Two raised beds were solarized for six weeks with a transparent and black polyethylene sheet, and a non-solarized (control) bed was equally made. The vegetable seeds were planted and their growths were recorded for four weeks. Soil samples at 15-20cm deep and leaves were obtained for microbiological and proximate analysis, respectively. The mean temperature of transparent xxpolyethylene's soil was 45.33 ^{o}C, black polyethylene (35 ^{o}C), and non-solarized (33.50 ^{o}C). The mean height and width of transparent polyethylene's *A. viridis* were 24 and 3cm, black polyethylene (19 and 2.2 cm), and non-solarized (17 and 1.6cm). The transparent polyethylene's soil had 3100 and 250 cfu/g bacterial and fungal colonies, black polyethylene (3200 and 1900 cfu/g), and non-solarized (37000 and 1900 cfu/g), respectively. The proximate contents of the transparent polyethylene's *A. viridis* were (70 moisture, 10 ash, 4.24 protein, 1.45 fat and 9.94 % fibre), black polyethylene (73.35 moisture, 8.36 ash, 3.1% protein, 1.23 fat, and 6.77 % fibre), and non-solarized (76.09 moisture, 5.91 protein, 3.15 ash, 1.31 fat and 6.75 % fibre). Overall, statistical differences ($p \leq 0.05$) existed between the solarized and non-solarized and between transparent and black polyethylene (transparent>black>non-solarized). Thus, solarization could be an effective strategy for controlling soil-borne bacteria and fungi of *A. viridis* in the area studied.

Keywords: *Amaranthus viridis, Environment, Pathogens, Polyethylene, Soil*

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INTRODUCTION

Amaranthus species is a popular group of vegetables that has about 70 members (Alegbejo, 2013). They are grown in the temperate and tropical climates of the world and are eaten as grains or vegetables (Alegbejo, 2013). The vegetable is highly nutritious and contains several phytochemicals, vitamins, and minerals (Peters and Gandhi, 2017). Amaranthus is anti-diabetic, anti-pyretic, anti-snake venom, anti-leprotic, anti-gonorrheal, anti-androgenic, anti-helmintic, anti-inflammatory, and has immunomodulatory properties (Alegbejo, 2013). In Nigeria and other sub-Saharan Africa, Amaranthus is considered the most consumed and traded green vegetable. It is a major source of nutrition in the region and provides income to farmers and market women. According to Ali *et al*. (2002), horticulture, which includes vegetable production, provides more employment per hectare of production than grain crop production. However, soil-borne pathogens constitute a great challenge to farmers in the region, reducing the nutritional content and market value of the vegetable. Some diseases of the vegetable include Alternaria rot (caused by *A. solani* and *A. tenuis*) and Phytophthora rot (caused by *P. infestans* and *P. nicotianae*) (Salau *et al*., 2012). Bacterial leaf spot (caused by *B. andyopogonis*) and leaf blight disease (caused by *R. solani*) are also frequently reported (Uppala *et al*., 2009). These pathogens are difficult to control because they can remain active for a long time in the host (Panth *et al*., 2020).

Common methods for managing soil-borne pathogens include the use of sanitation, resistant cultivars/varieties and grafting, cropping systems, soil solarization, bio-fumigation, and soil improvements (Panth *et al*., 2020). Others are anaerobic soil disinfestation, soil steam sterilization, soil fertility improvement, as well as chemical and biological control (Panth *et al*., 2020). In Nigeria and some other parts of the world, chemical control is the commonest. The chemical method is easy, fast, and effective, but harms human health and the environment and reduces plant pollinators and beneficial microorganisms (Christopher *et al*., 2010; Panth *et al*., 2020). An alternative strategy becomes imperative. Fortunately, in places with high sunlight and temperatures like Nigeria, solarization may be a suitable alternative.

Soil solarization, otherwise known as plasticulture, is an environment-friendly, pre-planting method that uses solar energy to control soil-borne pathogens, insects, and weed seeds (Mihajlovi´c *et al*., 2017). It is very effective for less extensive farming, such as irrigating vegetables and orchards. Soil solarization can be done by placing plastic sheets, most often transparent materials, over the seed bed after irrigation (Panth *et al*., 2020). Soil solarization can kill and alter microbial populations by heating the soil (Panth *et al*., 2020). In an experiment by Emoghene and Futughe (2011), *A. viridis plants* grown on solarized seed-beds grew higher and did not develop shoot disease compared with non-solarized. However, aside from sunlight and temperature mentioned earlier, some other factors also influence the performance of solarization. These factors include soil moisture, soil types, soil colour, soil structure and texture, and soil organic matter content (Katan, 1987; Panth *et al*., 2020). Other factors include the length of the day, the sensitivity of pathogens and pest species in the area to heat, cropping history, and other components of soil ecology (Katan, 1987; Panth *et al*., 2020). Thus, it is necessary to test the performance of soil solarization before its introduction in any area. Literature searches showed that such a study has not been conducted in Ikorodu, Lagos, Nigeria, and environs. Therefore, this study investigated the effects of soil solarization on fungal and bacterial populations associated with *Amaranthis viridis L*. (African spinach) in Lagos, Nigeria. The findings of the study will provide primary data for soil solarization application in the study area.

MATERIALS AND METHODS

DESCRIPTION OF THE STUDY AREA

This study was conducted in the Owode Ibese area of Ikorodu, Lagos State, Nigeria (Figure 1). Lagos State is in the Southwest of the country on latitudes 6 36'-38' N and longitudes 3 40'-3 42' 30' E (Salami *et al*., 2012). Lagos State borders Ogun State in the north and east, as well as the Republic of Benin in the west and the Atlantic Ocean in the south (Yahaya *et al*., 2020). The vegetation of the state is tropic with a short dry season between December and February and a long rainy season between March and November (Yahaya *et al*., 2020). The weather is very humid year-round, with monthly average maximum temperatures ranging from 28.6°C in July/August to 33.7°C in February/March (Ojeh *et al*., 2016). Ikorodu is on the outskirts of the state, characterized by extensive farmlands which are being rapidly lost to increasing urbanization. The soil of the study areas is composed of organic top soil, followed by loamy soil and lateritic sandy clay.

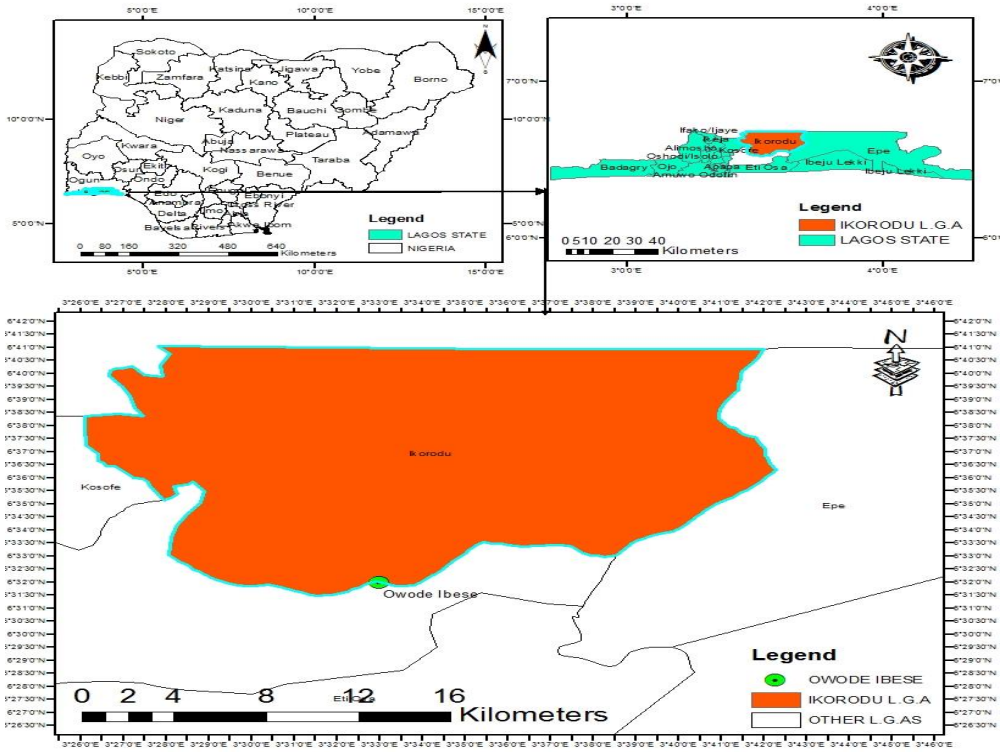

Figure 1: Location of Owode Ibese, Ikorodu, Lagos (ArcGIS 10.3 software)

COLLECTION OF A. VIRIDIS

Seeds of *A. viridis* were collected from a local farmer at Ikorodu in June 2020.

EXPERIMENTATION

The solarization was done as described by Elmore *et al.* (1997). A piece of land devoid of any debris was tilled, leveled, and moistened thoroughly for proper heat conductivity. Three raised planting beds (4 feet wide, 9 feet long, and 10 feet high) were made (Nair, 2016), of which one was covered with a black polyethylene sheet, transparent polyethylene sheet, and an uncovered planting bed served as the control. The polyethylene sheets were buried deep

around the bed to prevent them from flapping and tearing in the wind, which may have caused heat and moisture loss. The experiment was monitored daily and ensured that the beds were covered tightly with no holes in the polyethylene, which helped generate heat deep into the soil. After 6 weeks of solarization, the polyethylene sheets were removed and the *A. viridis* seeds were planted with a spacing of about 5 inches between seeds on all sides (Nair, 2016). The plant height and width of 20 samples of *A. viridis* from each bed were recorded weekly for four weeks. At the end of the experiment, 20 samples of the leaves of the *A. viridis* and 20 soil samples (at 15-20cm deep) from each bed were collected in sterile foil papers and taken to the laboratory for microbiological and proximate analysis, respectively.

PLANT HEIGHT AND WIDTH MEASUREMENT

The height and width of the *A. viridis* were measured as described by Moore (2018). The height was measured by placing a ruler on the ground next to the stem and measured to the height of the tallest stem. The width (diameter) was measured with a pair of calipers (Mitutoyo 500-196-20 Digital Vernier Caliper 150mm/6-inch Model).

SOIL TEMPERATURE MEASUREMENT

The soil temperature was measured between 1 and 2 pm daily, using a glass bulb thermometer as described by Sabri *et al*. (2018). A screwdriver was used to make a pilot hole in the soil to aid the insertion of the thermometer and avoid breakage. The thermometer was inserted 15cm deep into the soil (Elmore *et al*., 1997) and allowed to stay for about 5 minutes for the temperature to register.

MICROBIOLOGICAL ANALYSIS

The pour plate method described by Sanders (2012) was used to estimate the bacterial and fungal loads of the soil samples. Serial dilution was prepared by taking 1g of the soil in 9m1 of distilled water (diluent) in a sterilized test tube and shaken for several minutes to obtain a stock solution. One (1) ml was taken from the stock solution into 9ml of distilled water in a test tube, which constituted a 10^{-1} dilution. In the same manner, the second (10-^{2}), third (10^{-3}), fourth (10^{-4}), and fifth (10^{-5}) dilutions were prepared. One (1 ml) of each of 10^{-3}, 10^{-4}, and 10^{-5} dilution factors was placed in the centre of the Petri dishes using a sterile pipette, molten cooled nutrient agar and potato dextrose agar were then poured separately on the inoculum in the Petri dishes and the content mixed well. After solidification of the agar, the plates were incubated at 33°C (the average soil temperature) for 24 hours (nutrient agar) and 72 hours (potato dextrose agar). The potato dextrose agar was used to isolate fungi, while nutrient agar was used for bacteria isolates. The bacterial and fungal colonies grown in each media were then counted.

PROXIMATE ANALYSIS OF THE A. VIRIDIS

MOISTURE CONTENT

The moisture content of the vegetables was determined as described by AOAC (2000). Fresh leaves of the vegetables were weighed and oven-dried at 101°C for about 10 hours until a constant weight was obtained, after which it was cooled and re-weighed. The moisture content was obtained from the difference between the fresh and dry weight.

ASH CONTENT

The oven-dried samples obtained above were heated further in a muffle furnace at 550°C for about 3 hours. The ash content was obtained by subtracting the final weight from the initial weight.

CRUDE FIBRE CONTENT

The crude fibre content of the samples was obtained from the weight difference between the ash content and the sample analyzed (Ilodibia *et al*., 2014).

CRUDE FAT CONTENT

Five (5)g of each sample wrapped in filter paper was extracted in a Soxhlet apparatus using petroleum ether. The solvent was allowed to evaporate, after which the extracted material left was weighed and the fat content calculated.

CRUDE PROTEIN CONTENT

Crude protein was analyzed as described by the Kjeldahl method (AOAC, 2000). The samples were digested, distilled, and titrated. Using a conversion factor of 6.25 (Krul, 2019), the total nitrogen obtained was converted to crude protein. The percentage of protein in the samples was calculated thereafter.

DATA ANALYSIS

The Statistical Package for Social Science (SPSS) version 20 for Windows was used for all the analyses. Comparison of data among the test groups and control was done using ANOVA. Statistical significance was defined as $p \leq 0.05$.

RESULTS AND DISCUSSION

MICROBIAL COUNTS OF SOLARIZED AND NON-SOLARIZED SOILS

The bacterial and fungal counts of the solarized and non-solarized soil are presented in Table 1. Compared with non-solarized, the solarized soils had lower bacterial and fungal counts ($p \leq 0.05$). This result is consistent with Emoghene and Futughe (2011), who investigated the effects of solarization on *A. viridis* shoot disease and observed that only 5% of the plants in a solarized bed developed the shoot disease compared to 50% of the plants in non-solarized bed. Similarly, Zaid *et al*. (1991), showed that in a soil solarized for 45 days, fungal and bacterial populations were reduced by 76.6 and 71.1%, respectively. The reduction in the microbial populations of the solarized soils in this study, particularly soil solarized with the transparent sheet, could have been induced by the increased soil temperature following solarization (Table 1). According to Pokharel (2011), high temperatures raise soil volatile compounds to toxic levels and weaken soil microorganisms. Most organisms in the soil are negatively affected by temperatures greater than 39°C (Stapleton and DeVay, 1995), which was achieved by the soil solarized with transparent sheets in this study, but not in the other two soils (Table 1). This could explain why the soil solarized with transparent polyethylene had significantly ($p \leq 0.05$) lower bacterial and fungal counts than the soil solarized with black polyethylene. It further showed that, aside from trapping more heat, transparent polyethylene transmits heat in the soils more efficiently, leading to more microbial death. Transparent materials allow radiation to pass through and heat the underlying soil, while black materials absorb some solar energy and radiate it back (Krueger and McSorley, 2018). In 10-week soil solarization that compared the effectiveness of transparent and black polyethylene, the transparent material was slightly more effective (Abu-Gharbieh *et al*., 1991). Barakat (1987) also showed that a black material reduced soil temperatures by several degrees compared to a transparent material. However, Hasing *et al*. (2004) observed similar soil temperatures by transparent and black polyethylene following solarization.

Table 1: Total Viable Bacterial and Fungal Counts of Solarized and Non-solarized Soils in Ikorodu, Lagos Nigeria

Plot	Maximum Soil Temperature (°C)	Ambient Temperature (°C)	Bacteria count (cfu/g)	Fungal count (cfu/g)
Transparent Polyethylene Sheet	45.33±1.20*[a]	32.60±2.20	3150.00±28.9*[a]	258.33±4.14*[a]
Black Polyethylene Sheet	35.00±0.56	32.60±2.20	3250.00±27.9*	1883.30±44.1*
Control	33.50±0.50	32.60±2.20	37200.00±53	7817.00±58

Note: Values were expressed as means ± SD (n = 20); the values with an asterik (*) in the column are statistically different from the control at $p \leq 0.05$ (ANOVA); the values with a leter 'a' in the column are statistically different from black polyethylene sheet at $p \leq 0.05$ (ANOVA).

GROWTH PERFORMANCES OF SOLARIZED AND NON-SOLARIZED A. VIRIDIS

Table 2 contrasts the growth characteristics of *A. viridis* grown on solarized soils with those grown on non-solarized soils. Compared with the non-solarized, the *A. viridis* grown on the solarized soils significantly ($p \leq 0.05$) grew taller. This result is consistent with that of Sabatino *et al*. (2019), who reported higher plant growth on solarized soil compared with non-solarized. Some other studies also reported that black plastic materials resulted in higher early yields, but reduced total crop yield compared to transparent film (Schonbeck and Evanylo, 1998). As noted in Table 1, solarization increases soil temperatures, which, according to Farias-Larios *et al*. (1998), promotes nutrient availability and uptake by roots, resulting in increased growth. High temperatures also increase the activity of beneficial microorganisms, which speed up plant growth (Farias-Larios *et al*., 1998). Furthermore, solarization stabilizes temperatures up to 30cm deep in the soil, which favors root development and plant growth (Kasirajan and Ngouajio, 2012). However, the growth performance of plants under solarization depends on the capacity of light penetration or transparency, as evident in Table 2, in which plants grew significantly ($p \leq 0.05$) higher under transparent polyethylene than under black polyethylene. Helaly *et al*. (2017), monitored the effects of polyethylene types on the growth of husk tomato plants, and reported higher plant height, stem diameter, and leaf area by transparent polyethylene than black polyethylene. In another experiment that compared the effects of soil solarization among transparent, black, and white polyethylene sheets on lettuce yields, transparent polyethylene performed best (Mahmood *et al*., 2015). Solarization kills soil pathogens and pests. However, many beneficial soil organisms such as mycorrhizal fungi and *Bacillus species* can either survive solarization or recolonize the soil quickly afterward, improving soil nutrients, which aids plant growth (Stapleton *et al*., 2019).

Table 2: Growth Performances (Stem Length and Width) of *A. viridis* in Solarized and Non-solarized Soils in Ikorodu, Lagos Nigeria

Plot	Week 1 (cm)	Week 2 (cm)	Week 3 (cm)	Week 4 (cm)
Transparent Polyethylene Sheet	H= 2.87±0.18* W= 0.60±0.06	H= 9.63±0.09*[a] W=1.50±0.15	H=14.57±0.43*[a] W= 2.37±0.09*[a]	H=24.83±0.15*[a] W= 3.63±0.15*[a]
Black Polyethylene Sheet	H= 3.03±0.19* W= 0.70±0.12	H =6.83±0.04 W=1.00±0.06	H=10.73±1.23 W= 1.53±0.15	H= 17.63±1.11 W= 2.27±0.12*
Control	H = 2.35±0.05 W= 0.65±0.05	H = 5.87±0.26 W= 0.83±0.03	H = 9.67±0.24 W= 1.30±0.06	H= 16.40±0.61 W= 1.90±0.12

Note: Values were expressed as mean ± SD (n = 20); H = height; W= width; the values with an asterik (*) in the column are statistically different from the control at $p \leq 0.05$ (ANOVA), the values with a leter 'a' in the column are statistically different from black polyethylene sheet at $p \leq 0.05$ (ANOVA).

PROXIMATE COMPOSITION OF THE SOLARIZED AND NON-SOLARIZED A. VIRIDIS

The moisture, ash, crude protein, fat, and the crude fibre content of the *A. viridis* solarized with transparent and black polyethylene sheets as well as non-solarized are presented in Table 3. The *A. viridis* grown on soil solarized with the transparent polyethylene sheet had the highest levels ($p \leq 0.05$) of ash, crude protein, fat, and crude fibre, while moisture content was highest in the control. Generally, there was no significant difference ($p \geq 0.05$) between the *A. viridis* solarized with the black polyethylene sheet and the non-solarized in the levels of the mentioned nutrients. These results follow similar trends with the phytonutrient properties of some solarized plants compared with the non-solarized. Notably, Grünzweig *et al*. (1999) reported increased concentrations of nitrogen and copper in solarized tomato plants compared with the non-solarized tomatoes of the same species. In another study, solarization with calcium cyanamide significantly increased the ascorbic acid and phenolic content of tomato fruits compared with the non-solarized (Sabatino *et al*., 2019).

The high proximate compositions of the *A. viridis* solarized with a transparent polyethylene sheet showed that solarizing with the material promotes plant nutrient accumulation. According to Saloum and Almahasneh (2015), soil solarization improves soil physical, chemical, and biological properties, resulting in improved overall plant quality, including nutritional contents. Soil solarization boosts the bioavailability of phytochemicals and phytonutrient-enhancing soil nutrients such as nitrogen, calcium, and magnesium as well as extractable phosphorus and potassium (Pokharel, 2011).

Table 3: Proximate Composition of Solarized and Non-solarized *A. viridis* Grown in Ikorodu, Lagos Nigeria

Plot	Moisture Content (%)	Ash Content (%)	Crude Protein (%)	Fat Content (%)	Crude Fibre (%)
Transparent Polyethylene Sheet	70.98±4.24*[a]	10±2.0*[a]	4.24±0.00*[a]	1.45±0.57	9.94±0.00*[a]
Black Polyethylene Sheet	73.35±0.27*	8.36±1.9	3.16±0.28	1.23±0.06	6.77±1.1
Control	76.09±0.01	8.31±0.11	3.35± 0.15	1.31±0.45	6.75±0.11

Note: Values were expressed as means ± SD (n = 20); the values with an asterik (*) in the column are statistically different from the control at $p \leq 0.05$ (ANOVA); the values with a leter 'a' in the column are statistically different from black polyethylene sheet at $p \leq 0.05$ (ANOVA).

LIMITATIONS OF THE STUDY

Due to financial constraints, the study could not investigate the effects of solarization on many microorganisms associated with *A. viridis*. For the same reason mentioned, the study could not also assess the performance of seed bed covers other than transparent and black polyethene.

CONCLUSION

The results showed that the solarized soils had lower bacterial and fungal counts than the non-solarized, which suggests that solarization had killed some soil microorganisms. The transparent polyethylene sheet reduced the microbial counts more than the black polyethylene because the transparent material trapped and transmitted solar energy more efficiently. Moreover, the *A. viridis* planted in the solarized soils grew more in height and width than in the non-solarized soil. The positive effects of soil solarization on the *A. viridis* are evident in their proximate compositions, in which the *A. viridis* in the transparent and black polyethylene (in that order) had higher fat, crude fibre, protein, and ash content. Overall, findings from this study imply that solarization may be an effective strategy for controlling soil-borne pathogens of *A. viridis* in the studied area. We recommend similar studies on other plants grown in the area and other regions in Nigeria.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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