IMPACT OF SOIL SOLARIZATION ON AMARANTHUS VIRIDIS AND MICROBIAL POPULATION

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ABSTRACT

The impact of solarization on the growth of *Amaranthus viridis* and soil microbial population was carried out in a plot. After solarization, soil maximum temperature was 48.67°C at 5cm depth and 40.00°C at 10cm depth in solarized and unamended bed (C). The heterotrophic bacteria population showed increase in post-solarization with a mean count of $2.98x10^6$cfu/g as compared to pre-solarization with a mean count of $2.25x10^6$cfu/g in solarized and amended bed (A). *Bacillus subtillis, Pseudomonas* sp. *Escherichia coli, Enterobacter* sp. and *Micrococcus varians* were isolated from both pre- and post-solarized bedss. Population of fungi in all beds appear to be more in the post-solarization than in pre-solarization. Post-solarization of solarized and amended plot (A), showed a total mean count of $3.24x10^6$cfu/g compared to a total mean count of $1.98x10^6$cfu/g in pre-solarization. The fungi commonly isolated from both pre- and post-solarization were *Aspergillus niger, A. flavus, A. fumigatus, A. tamari, Penicillium notatum, P. chrysogenum, Mucor* sp., *Rhizopus stolonifer* and *Trichoderma viridis* which was higher in post-solarization while *Mycosphaerella fijiensis, Cladosporium* sp. and *Helminthosporium* sp. were found only in pre-solarization. In solarized and amended bed (A), *Amaranthus viridis* grew to a mean height of 104.69cm from an original mean height of 2.91cm while non-solarized and amended bed (B), *A. viridis* grew to a mean height of 41.86cm from an original mean height of 2.91cm. In solarized and unamended bed (C), *A. viridis* grew to a mean height of 94.27cm from an original mean height of 2.89cm. However, in the non-solarized and unamended bed (D), *A. viridis* showed the least with a mean height of 36.60cm at the end of the experiment from an original mean height of 2.91cm. Solarization had significant effect on the heights of *Amaranthus viridis* even at higher probability level ($P<0.0005$).

INTRODUCTION

Soil solarization is a process in which soil temperature is increased by using solar radiation as an energy source. It was initially intended as a method for controlling soil pathogens (Katan *et al*., 1976) but research has shown that it has other effects on soil characteristics that can influence the performance of crops, such as nutrient concentration (Chen *et al*., 1991) and soluble organic matter content.

Soil solarization often enhances plant growth and yield in pathogen-free soils. Noto (1994) reported higher yields and reduced nematode damage for tomato plants grown on solarized soil, compared to those planted in non-treated soil. Abd El-Megid *et al*. (1998) documented increased plant growth of onion transplants produced in solarized seedbeds, apparently without incidence of diseases. These reports correspond to a phenomenon known as increased growth response (IGR) that has been attributed to several mechanisms, including increases in nutrient levels in the soil solution, stimulation of beneficial organisms and control of minor pathogens (Gruenzweig *et al*., 1993).

Solarization creates a partial biological vacuum in the soil. Although heat tolerance varies among organisms, generally only minutes are required at temperatures above 45ºC to reach LD90 levels (Stapleton, 1991). However, populations of mesophilic organisms decline at faster rates during solarization. For these organisms, accumulation of heat effects

above 37ºC over time is lethal (DeVay, 1991). Thermo tolerant and thermophilic organisms usually survive the solarization process, but become weakened and vulnerable to changes in their ecosystem. Most plant pathogens and pests are mesophilic, being unable to grow at temperatures above 31-32ºC (DeVay and Katan, 1991).

Environmental factors other than temperature can also affect the sensitivity of organisms to heat, altering their thermal death curves. Besides influencing soil heat conductivity, moisture stimulates the metabolic activity of organisms in the soil, rendering them more susceptible to thermal inactivation (Egley, 1990; Stapleton, 1991). Organisms are more tolerant to heat exposure when dormant or inactive under dry conditions (Elmore, 1998).

Amaranthus spp belong to the family Amaranthaceae, rightly described as vegetables which are the fresh edible part of plants namely the leaves, stems, fruit seeds, pulses and spices (Odebunmi-Osikanlu, 1977). In Nigeria and many other parts of West Africa, the leaves as well as the succulent young stems of *Amaranthus* spp. especially *A. hybridus* are consumed as vegetables (Ikediugwu *et al.,*. 1994) and are reported by Oyenuga (1968) to be rich in proteins, vitamins and minerals. In Nigeria, there is dearth of information on soil solarisation. This study therefore aims at conducting field experiments to evaluate the impact of solarisation on the growth of *Amaranthus viridis* and soil microbial population.

MATERIALS AND METHODS

Site Description, soil particle size and pH determination

The experimental field plot was located beside Animal House, Faculty of Life Sciences, University of Benin, Nigeria. Field experiments were conducted from June to September, 2010. The soil particle size of sand (84.32%), silt (2.70%) and clay (12.98%) was determined following the method of Chen *et al*. (1991). One hundred grammes (100g) of soil was weighed into 1 litre-shaking bottle, 20ml Calgon, 3ml 1N NaOH and 200ml of water were added; and shaken on an electric shaker for 3 hours. This was then removed, transferred quantitatively to the mechanical analysis cylinder and water was added to make up the volume to the first 113ml mark. The cylinder was shaken by inverting it a few times, placed on the laboratory bench and after 4½ minutes, hydrometer was inserted and after 5 minutes, the scale was read. The pH 6.33 was obtained essentially employing the method of Conn and Lazarovits (1999). Soil (3g) from experimental field was added to a tube containing 15ml of distilled water and shaken at maximal speed by electric shaker for 30 seconds. The soil suspension was incubated at 25^{o}C for 30 minutes and pH value was then determined with a digital pH meter (Jenway 3020).

Experimental Design

Experimental plot consisted of four rows arranged in a randomized complete block design with four replicates at four different blocks per treatment in a seed-bed. *Amaranthus viridis* seedlings from nursery soil amended with poultry manure in wooden crate and were transplanted into the experimental plots after 3 weeks. The treatments applied were,

i. Solarized beds amended with poultry manure (**A**),
ii. Non-solarized beds amended with poultry manure (**B**),
iii. Solarized and un-amended beds (without poultry manure) (**C**) and
iv. Non-solarized and un-amended beds (without poultry manure) (**D**).

Each main bed measured approximately 1.5 x 2.5 m^2. Prior to the treatments, the soil was tilled manually. Field beds were raised; they were 25 cm in height with 50 cm between rows and irrigated to field capacity to a depth of 50 to 60 cm. Transparent polyethylene sheeting with 0.02µm thickness was laid over raised randomly selected field beds and the open edges of the polyethylene sheeting was anchored to the soil by burying the edges in a shallow trench around the treated area (Plate 1). Polyethylene sheets were covered during June-July for 7 weeks and left in place throughout the growing season, providing the benefits of both soil solarization and mulching.

Soil Temperature

Soil temperatures were measured 5 and 10 cm below the top of the bed for each treatment. This was done during the afternoon by piercing the soil with mercury in glass thermometers and the temperatures were recorded three times a week (Mondays, Wednesdays and Fridays).

Amaranthus viridis

The seeds of *Amaranthus viridis* used in this research work were obtained from the Department of Crop Science, Faculty of Agriculture, University of Benin. A wooden crate containing a 2:1 mixture of garden soil and Chicken manure was used as a seed bed for growing the *A. viridis* in green house according to Ikediugwu *et al*. (1994). The seedlings were transplanted when three weeks old from the wooden crate onto the four treatment beds with their four replicates (A_1 - A_4, B_1 - B_4, C_1 - C_4 and D_1 –D4 (Plate1). Bed A was solarized and amended, bed B was not solarized but amended, bed C was solarized but unamended and bed D was not solarized and unamended. Slits cut were made on the transparent polyethylene sheets for transplanting of the seedlings were applicable (Plate 2). The planting spacing was maintained at 15cm by 15cm with a uniform plant density of 30 *A. viridis* seedlings per bed.

Plate1. *The field site, arranged in a randomized complete block design with four replicates at four different blocks per treatment.*

A= SOLARIZED AND AMENDED BED **(A)**

B= NON-SOLARIZED AND AMENDED BED **(B)**

C= SOLARIZED AND UNAMENDED BED **(C)**

D=NON-SOLARIZED AND UNAMENDED BED **(D)**

Note: Subscript numbers represent replicates of each treatment.

Plate2. *The seedlings were transplanted when three weeks old from the wooden crate onto the four treatment beds with their four replicates.*

Measurement of Growth Parameters

Plant height, diameter of shoot, mean length and width of leaves, number of leaves and root length were measured from 240 randomly selected *Amaranthus viridis* plants from the 16 beds. Fifteen (15) out of the 30 *A. viridis* plants per bed were randomly selected, marked and their growth rate determined with a measuring tape. Root length from the base of the stem to the longest root tip was measured at the end of the experiment.

Collection of Soil Samples

Soil samples were collected before and after solarization to determine the micro flora of the soil. All soils were sampled to 10cm depth into the soil of the randomized complete block design with four replicates at four different blocks per treatment as shown in Plate 1. The soil samples were transported in different sterile polythene bags to avoid cross-contamination to laboratory for microbiological analysis.

Determination of Microorganisms from Soil Samples

For isolation purpose, the soil dilution method was used. Samples collected from all sites in each case were mixed together before making dilution. One gramme of soil was weighed after mixing properly. This soil was placed in test-tube containing 9ml of sterile water. The test-tube was placed and shaken on an electric shaker at high speed for one minute to give a 10^{-1} dilution. Waited for one minute to allow the soil particles to settle down which was used as stock sample and serial dilution of 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} were prepared for each of the four treatments with their four repli-

cates.

Pour plates of each of the serial dilution were prepared using approximately 15ml of Nutrient Agar and Potato Dextrose Agar and 1.0ml of the serial dilution suspension for bacteria and fungi respectively. Two replicates pour plates for each of dilutions were incubated at 37^{o}C for 24 hours before the isolation of bacteria and a minimum of 72 hours before fungal culture incubated on a laboratory bench under a five-foot fluorescent day light tube at room temperature (28^{o}C ± 2^{o}C) were isolated. Pure cultures of fungal and bacterial isolates were characterized with reference to standard texts of Barnett and Hunter (1972), Buchanan and Gibbon (1974) respectively.

Isolation and Characterization of Heterotrophic Bacteria from Soil Sample.

Distinct colonies that appeared on each Petri dish after incubating at 37^{o}C for 24 hours were counted and the colony forming units per gram (CFU/g) was determined. Pure culture was made from each distinct colony. The bacterial isolates were characterized on the basis of their cultural, morphological and biochemical characteristics.

Isolation and Characterization of Fungi from Soil Samples.

Colony forming unit per gram (CFU/g) of fungal isolates were counted and pure cultures were identified. Identification was based on cultural and morphological characteristics of isolates grown on Potato Dextrose Agar with reference to the manual of Barnett and Hunter (1972). Visual observation of the agar cultures was made periodically and the fungus and surrounding agar, especially of the underside, surface texture and the presence of macroscopic structures were noted. Further observations were made on slide mounts. The mounts were made both in distilled water and lactophenol cotton blue. A dissecting needle was used to remove some portions of the fungus and placed on two separate slides, one with a drop of water and the other with the lactophenol cotton blue. The fungal fragments were then teased until thinly spread after which a cover slip was placed on top and observed under light microscope with X10 and X40 objectives.

RESULTS AND DISCUSSION

The maximum soil temperature averages were 47.88^{o}C at 5cm depth and 40.83 ^{o}C at 10cm depth in solarized and amended beds (**A**). However, the maximum soil temperature averages were 39.67^{o}C at 5cm depth and 37.50 ^{o}C at 10cm depth in non-solarized and amended beds (**B**). Solarized and un-amended beds (**C**) had maximum soil temperature averages of 48.67^{o}C at 5cm depth and 40.00^{o}C at 10cm depth, while non -solarized and un-amended beds (**D**) had a maximum soil temperature average of 39.67^{o}C at 5cm depth and 35.58 ^{o}C at 10cm depth (Figures 1 and 2). Soil temperatures at a depth of 5cm were approximately 8^{o}C higher in solarized plots compared with non-solarized plots. Furthermore, soil temperature showed no significant difference between solarized amended and solarized un-amended beds.

The heterotrophic bacteria isolated, characterized and identified from the various sampling beds are given in Figure 3. Generally, bacterial population showed increase in post-solarization with a mean count of 2.98x10^{6}cfu/g as compared to pre-solarization with a mean count of 2.25x10^{6}cfu/g in solarized and amended bed (A). Solarized and unamended bed (C) showed a mean count of 1.99x10^{6}cfu/g in post-solarization compared to a mean count of 1.55x10^{6}cfu/g in pre-solarization. *Bacillus subtillis, Pseudomonas* sp. and *Micrococcus varians* were isolated from all the pre-solarized plots (Table 1). *Escherichia coli* and *Enterobacter* sp. were commonly found in the amended beds with chicken manure in pre-solarized beds. The post-solarized plots contained *Bacillus subtillis* and *Pseudomonas* sp. in higher quantity.

Table 1: Synopsis of microbial isolates from pr- and post-solarized soil of an experimental plot used in growing *A. viridis*

Soil sample	Microorganisms isolated/identified
Pre-solarized soil	*Bacillus subtillis, Pseudomonas* sp. *Micrococcus varians, Aspergillus niger, A. flavus, A. fumigatus, A. tamari, Penicillium notatum, P. chrysogenum, Mucor* sp., *Rhizopus stolonifer*, *Trichoderma viridis*, *Mycosphaerella fijiensis, Cladosporium* sp. and *Helminthosporium* sp.
Post-solarized soil	*Bacillus subtillis*, *Pseudomonas* sp, *Aspergillus niger, A. flavus, A. fumigatus, A. tamari, Penicillium notatum, P. chrysogenum, Mucor* sp., *Rhizopus stolonifer*, *Trichoderma viridis*

Population of fungi in all beds appeared to be more in the post-solarization than in pre-solarisation. Solarized and amended bed (A) showed a total mean count of $3.24x10^6$cfu/g compared to a total mean count of $1.98x10^6$cfu/g in pre-solarization. Solarized and unamended bed (C) had a total mean count of $2.69x10^6$cfu/g at post-solarization compared to 0.9Population of fungi in all beds appeared to be more in the post-solarization than in pre-solarisation. Solarized and amended bed (A) showed a total mean count of $3.24x10^6$cfu/g compared to a total mean count of $1.98x10^6$cfu/g in pre-solarization. Solarized and unamended bed (C) had a total mean count of $2.69x10^6$cfu/g at post-solarization compared to $0.95x10^6$cfu/g at pre-solarization (Figure 4). The fungi commonly isolated from both pre- and post-solarization were *Aspergillus niger, A. flavus, A. fumigatus, A. tamari, Penicillium notatum, P. chrysogenum, Mucor* sp., *Rhizopus stolonifer* and *Trichoderma viridis* which were higher in post-solarization while *Mycosphaerella fijiensis, Cladosporium* sp. and *Helminthosporium* sp. were found only in pre-solarization (Table 1). Non-solarized and unamended soil (D) had the lowest fungal count ($1.29\ x10^6$ cfu/g) compared to the highest count of solarised and amended soil (A) with $3.24\ x10^6$ cfu/g (Figure 4).

In solarized and amended bed (A), *Amaranthus viridis* grew to a mean height of 104.69cm (9^{th} week) from 2.91cm (3^{rd} week during transplanting). The result showed a cumulative increase in height of 101.78cm with growth rate of 2.42cm/day. Non-solarized and amended bed (B), *A. viridis* grew to a mean height of 41.86cm (9^{th} week) from 2.91cm (3^{rd} week). It also showed a cumulative increase in height of 38.95cm and a growth rate of 0.93cm/day. In solarized and unamended bed (C), *A. viridis* grew to a mean height of 94.27cm (9^{th} week) from 2.89cm (3^{rd} week) with a cumulative increase in height of 91.38cm and a growth rate of 2.18cm/day. However, in the non-solarized and unamended bed (D), *A. viridis* showed the least with a mean height of 36.60cm at the end of the experiment (9^{th} week) from 2.91cm when transplanted (3^{rd} week), with a cumulative increase in height of 33.69cm and a growth rate of 0.80cm/day (Figure 5 and Plate 3).

The mean diameter of shoot of *A. Viridis* was highest in solarized and amended bed (5.65cm) and lowest in non-solarized and unamended bed (2.81cm)(Figure 6). At the end of the experiment (9 weeks), the mean lengths of leaves of *A. viridis* were 30.19cm, 19.50cm, 32.52cm and 17.34cm for solarized and amended bed (A), non-solarized and amended bed (B), solarized and unamended bed (C) and non-solarized and unamended bed (D) respectively (Figure 7).

Also the mean widths of the leaves of *A. viridis* were 9.57cm, 6.65cm, 8.87cm and 5.43 cm in solarized and amended bed (A), non-solarized and amended bed (B), solarized and unamended bed (C) and non-solarized and unamended bed (D) respectively (Figure 8).

The mean number of leaves of *A. viridis* in solarized and amended bed (A), was 137.69cm while non-solarized and amended bed (B), which was the least was 31.44cm. In solarized and unamended bed (C), the mean number of leaves is 142.28 which was the highest and non-solarized and unamended bed (D) had 44.34 (Figure 9). The mean length of *Amaranthus viridis* roots are 49.75cm, 36.25cm, 46.50cm and 30.00cm in solarized and amended bed (A), non-solarized and amended bed (B), solarized and unamended bed (C) and non-solarized and unamended bed (D) respectively. $5x10^6$cfu/g at pre-solarization (Figure 4). The fungi commonly isolated from both pre- and post-solarization were *Aspergillus niger, A. flavus, A. fumiga-*

tus, A. tamari, Penicillium notatum, P. chrysogenum, Mucor sp.*, Rhizopus stolonifer* and *Trichoderma viridis* which were higher in post-solarization while *Mycosphaerella fijiensis, Cladosporium* sp. and *Helminthosporium* sp. were found only in pre-solarization (Table 1). Non-solarized and unamended soil (D) had the lowest fungal count (1.29 x10^6 cfu/g) compared to the highest count of solarised and amended soil (A) with 3.24 x10^6 cfu/g (Figure 4).

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Plate3. *The growth responses of matured Amaranthus viridis at nine weeks old on the four treatment beds with their four replicates.*

Amaranthus viridis grew better under field condition in solarized plots which were covered with transparent polyethylene sheets, as demonstrated by increased in height of shoot, length and with of leaves, number of leaves and root length. In solarized and amended beds (A), a height (104.69cm) was obtained as against 41.86cm obtained in non-solarized and amended beds (B). Solarized and unamended bed (C), had a height of 94.27cm against 36.60cm of non-solarized and unamended beds (D) .

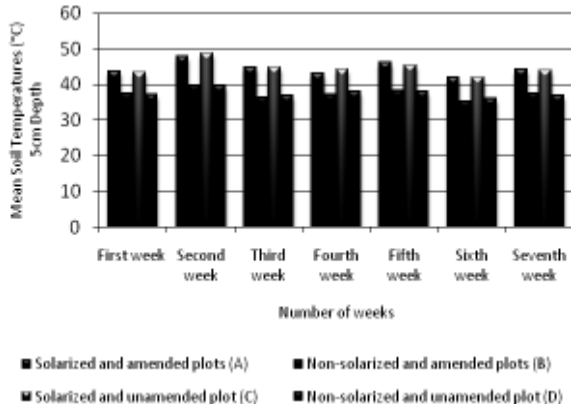

Figure1. *Mean Soil Temperatures (°C) During Solarization from June to July (7 weeks) 2010 at 5 cm depth of experimental field.*

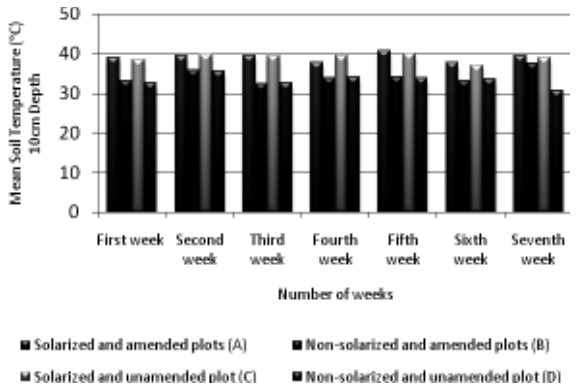

Figure2. *Mean Soil Temperatures (°C) During Solarization from June to July (7 weeks) 2010 at 10 cm depth of experimental field,*

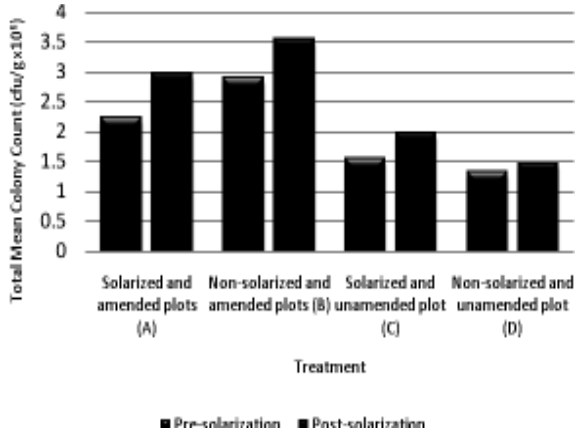

Figure3. *Total Heterotrophic Bacteria Count (cfu/g x 10^6) of Soil Samples in Pre and Post- Solarization*

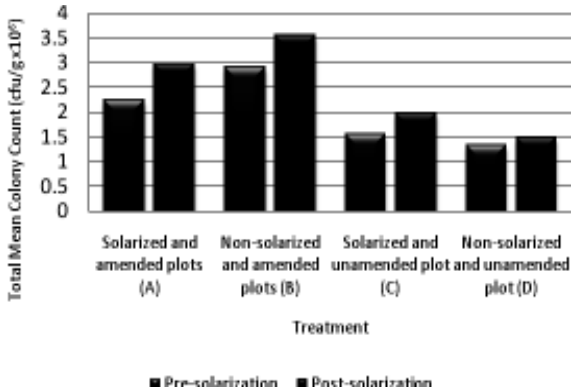

Figure4. Total fungi Count (cfu/g x 10^6) of Soil Samples in Pre and Post- Solarization

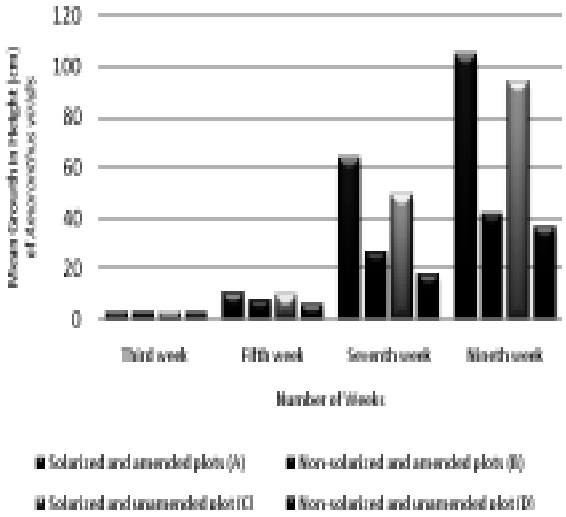

Figure 5. *Mean Height (cm) of Amaranthus viridis under Field Condition from July to September(6 weeks) 2010.*

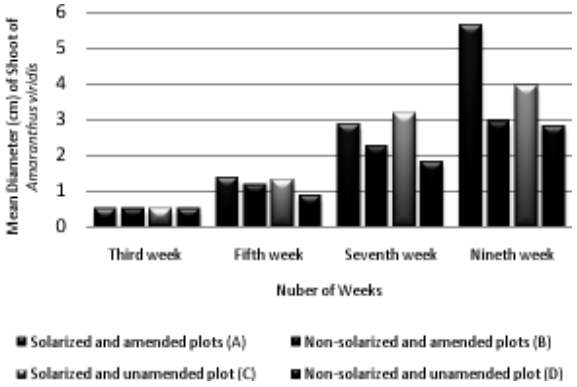

Figure 6. *Mean Diameter (cm) of Shoot of Amaranthus viridis under Field Condition from July to September (6 weeks) 2010*

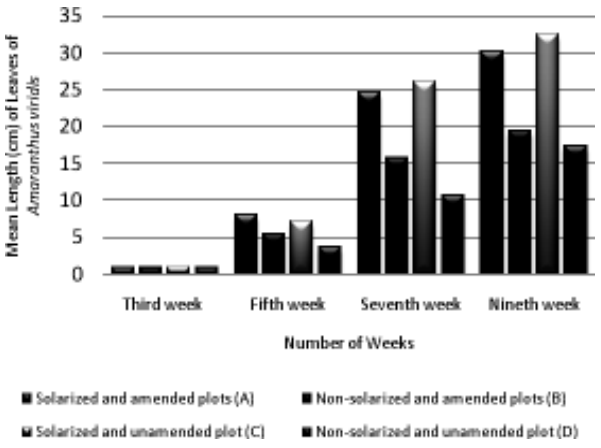

Figure 7. *Mean Length (cm) of Leaves of Amaranthus viridis under Field Condition from July to September(6 weeks) 2010.*

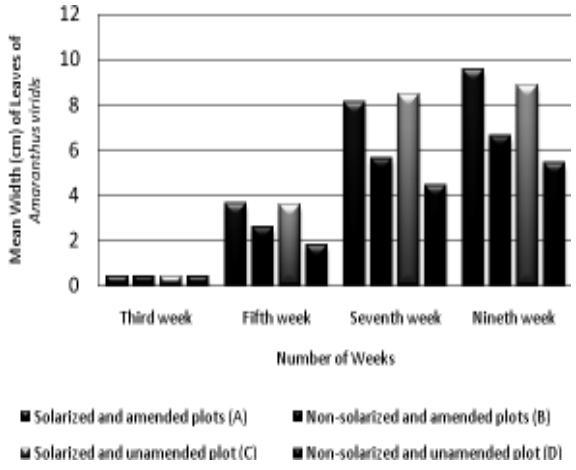

Figure 8. *Mean Width (cm) of Leaves of Amaranthus viridis under Field Condition from July to September (6 weeks) 2010.*

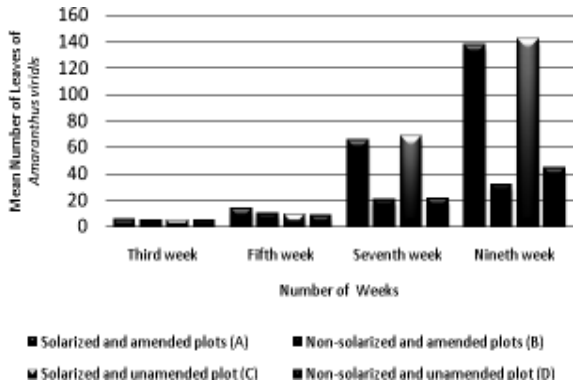

Figure9. *Mean Number of Leaves of Amaranthus viridis under Field Condition from July to September (6 weeks) 2010.*

Reports of the effects of solarization on culturable soil fungal counts are variable. Increases and decreases, as well as no changes have been reported (Chen *et al.,* 1991). Fungal counts from soil are difficult to estimate reliably because of soil heterogeneity and culturing bases. The findings of this research were consistent with those of Stapleton (1991) and Katan *et al.* (1976), that solarization generally increased total fungal propagule counts in soil which was also true for heterotrophic bacteria.

CONCLUSION

With the increasing environmental awareness of public health, soil solarization has no health or safety problems associated with use; crops produced are pesticide-free and may command a higher market price.

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