Effective Microorganisms and their influence on growth and yield of pigweed (Amaranthus dubians)

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EFFECTIVE MICROORGANISMS AND THEIR INFLUENCE ON GROWTH AND YIELD OF PIGWEED (*Amaranthus dubians*)

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ABSTRACT

Pigweed (*Amaranthus dubians)* plants grow widely in many parts of the world. It is a very popular indigenous wild vegetable and is usually utilized by many communities as a nutritional additive. Pigweed is becoming very popular nowadays because of its high nutritive value and simple growth habits. However there is very little information about growth of this plant, using effective microorganisms, thus this study is designed to evaluate the effect of inoculation of effective microorganism on growth and yield of pigweed. The experiment was performed in five liter pots representing various conditions in the field. It comprised of four treatments, replicated, five times and arranged in a complete randomized design. One set of the treatments consisted of soil collected from the field, while the other treatments consisted of soil and organic manure prepared using effective microorganisms, sterilized soil treated with effective microorganisms, and sterilized soil plus organic manure without application of effective microorganisms respectively. Shoot height, stem diameter, leaf number per plant, leaf area, leaf fresh weight, leaf dry weight, root fresh weight, root dry weight and chlorophyll a and b contents were determined. Inoculated pigweeds with effective microorganisms recorded highest values in all the parameters measured except the root dry matter accumulation. There were significant differences ($p \leq 0.05$) in shoot height growth, stem diameter, leaf numbers per plant, leaf area, leaf fresh and dry weight and root fresh and dry weights among treatments. There were no significant differences in leaf chlorophyll content among the treatments even though chlorophyll *a* and *b* contents were slightly higher in plants inoculated with effective microorganisms. The three other treatments had significantly lower values of the parameters determined. The results from this study demonstrated that growth and yield of pigweeds may be improved by inoculating the plants with effective microorganisms, and as a result reduce the use of fertilizers in production of this vegetable hence promoting sustainable agriculture. More studies would be needed to determine the effects of effective microorganisms' inoculation on other amaranthus species.

Keywords: pigweed, chlorophyll content, effective microorganisms, growth, yield, plant nutrition.

INTRODUCTION

Environmental protection is getting more important for the agrarian, because of the purpose of sustainable agriculture. It is an important issue whether the replacement of organic fertilizers and chemical fertilizers with biofertilizers causes a beneficial increase in dry weight (Lévai *et al*., 2006). Nitrogen and phosphorus are essential nutrients for plant growth and development. Application of organic matter positively affects the growth and development of plant roots and shoots (Ghosh *et al*., 2004). There is evidence from the literature that application of biofertilisers can help to convert nutritionally important element from unavailable form to available form through biological processes. Organic fertilizers are good sources of nutrients for crop production and improving physical and chemical properties of soil. Microorganisms are important attributes in agriculture to promote the circulation of plant nutrients and reduce the need for chemical fertilizers. Biofertilizers are organic products containing living cells of different types of microorganisms that have emerged as important component of the integrated nutrient supply system and hold a great promise to improve crop yields through environmentally better nutrient supplies. These non infecting plant growth promoting bacteria (PGPB) might affect mineral nutrition of plants through their influence on: i) growth, morphology and physiology of roots; ii) the physiology and development of plants; iii) the availability of nutrients; and nutrient uptake processes. A lot of microorganisms, for example species of Bacillus and Pseudomonas have a direct effect on the plant growth (Kloepper *et al*., 1986). The concept of effective microorganism, (EM) was developed by Professor Teruo Higa University of the Ryukyu, Okinawa, Japan (Higa, 1991). Effective microorganisms (EM) consist of mixed cultures of beneficial and naturally occurring microorganisms that can be applied as inoculants to increase the microbial diversity of soil and plant. Research has shown that the inoculation of EM cultures to the soil plant ecosystem can improve soil quality, soil health and the growth, yield and quality of the crops (Kengo and Hui-lian, 2000).

Decline in soil fertility, increased soil erosion and increasing shortage of food are major factors affecting human health in Africa. Fertilizers are costly and therefore out of reach of most resource poor farmers. There is also increasing evidence that synthetic agrochemicals and fertilizers have caused adverse effects on the environment leading to loss of biodiversity. This observation has promoted the need of introducing methods of farming aimed at reducing health risks including the use of effective microorganisms in organic farming. Inoculation of crops with effective microorganisms can improve vegetable crop yields and there by improve food security. Although pigweed is a very important indigenous vegetable in Kenya, there is hardly any information from the literature on the cultivation of pigweed using effective microorganisms.

Amaranth species are cultivated and consumed as leafy vegetables in many parts of the world (Schippers, 2000). Amaranth greens, are common leafy vegetables throughout the tropics and in many warm temperate regions .The vegetables are very good sources of vitamins such as vitamin A, vitamin B6, vitamin C, riboflavin, and folate, and dietary minerals including calcium, iron, magnesium, phosphorus, potassium, zinc, copper, and manganese. Because of its valuable nutrition, some farmers grow amaranth today. Traditional green vegetables occupy an important role in household nutrition throughout the world particularly in rural areas. Indigenous vegetables such as pigweed can contribute substantially to food security in the developing world and therefore need to be fully integrated in our farming systems. The vegetables are very rich in vitamins and proteins and, can help boost immunity amongst the HIV victims, improve the health of infants and breastfeeding mothers.

Effective microorganisms can contribute significantly to the production of indigenous vegetables such as pigweeds. Understanding and use of effective microorganisms in vegetable production in Kenya is limited (Muthaura, Personal communication). Data is lacking from the literature review to justify the use of effective microorganisms in cultivation and production of pigweeds in Kenya. The main objective of this study was to investigate the influence of effective microorganisms on growth and yield of pigweed (*Amaranthus dubians)*. It was hypothesized that organic manure prepared using effective microorganisms improves growth and yield of pigweed.

MATERIALS AND METHODS

Compost preparation using effective microorganisms

A mixture of EM-1 (1 ml L^{-1}), molasses and water was sprayed on rice straw and left to stand for about three weeks. The resultant manure was used as a media for pigweed propagation.

Experimental materials and growth conditions

This experiment was conducted at Botanical Garden, Maseno University, Kenya. Seeds were acquired from the Botanical Garden of Maseno University. Ten viable seeds were sown in 5 liter plastic pots (20 cm diameter and 30 cm high), containing about 2.5 kg of sterilized soil (classified as kandiudalfic Eutrodox (Musyimi *et al*., 2007). The seedlings that emerged were thinned to two plants per pot, ten days after germination. The pots were watered on daily basis to maintain the soil moisture content at approximately 60% water holding capacity.

Experimental designs and treatments

The experimental design was a completely randomized design with four treatments: Soil plus manure containing effective microorganisms (A), soil only (B), Soil plus manure only (C), and soil plus effective microorganisms without manure (D). The treatments were replicated five times and the pots containing the seeds were arranged inside a green house whose conditions were: Temperature: min/max 20/41^{0}C and relative humidity: min/max 50/95% (Plate-1). Weeds were controlled by hand pulling. Watering was carried out on daily basis. After germination the seedlings were thinned out leaving only two plants per pot. Data collection commenced fourteen days after seed germination and seedling establishment.

Plate-1. Experimental layout in the greenhouse.

Measurement of growth parameters

Growth measurements commenced fourteen days after seed emergence and seedling establishment. Data was collected at an interval of four days up to the end of the experiment. Shoot height was measured using a meter rule. The number of fully expanded mature leaves was established by counting. Leaf area expansion was determined at the end of the experiment according to Jose *et al.* (2000).

$A_L = 0.73 (L_L X W_L)$, where L_L is the leaf length and W_L is the maximum width measured for all leaves on each plant. The stem diameter was determined at the end of the study by use of a vernier caliper. At maturity, the plants were harvested and their roots, shoots and leaves separated. Roots were washed in tap water after soaking, blotted dry on paper towels and weighed using an electronic balance. Fresh weight reading for the roots shoots and stems were taken immediately after harvesting. All the plant samples were oven dried at 60 ^{0}C to constant dry weight for at least 48 hours after which their dry weight was taken using a weighing balance.

Determination of chlorophyll content

The fifth leaf from the shoot apex from each plant in each treatment was collected for chlorophyll extraction and concentration determination. Chlorophyll concentration was determined in 80% acetone extract by use of a spectrophotometer. Absorbency was measured against an 80% acetone blank at 647nm and 664nm. The values of chlorophyll a, chlorophyll b was determined according to Yadegari *et al.* (2007) using the following formula:

Chlorophyll *a* = 13.19A664-2.57A647 (mg/g fresh weight)
Chlorophyll *b* = 22.1 A647-5.26 A664 (mg/g fresh weight)

Where, A664 was absorbance at 664nm and A647 was absorbance at 647nm, respectively.

Data analysis

Data collected was analyzed using SAS statistical computer package. Analysis of variance (ANOVA) was carried out to determine whether there were any significant differences among treatments on parameters measured.

RESULTS

Shoot height

Shoot growth occurred in all the treatments over the experimental period. There were significant differences in shoot growth among the treatments ($P \leq 0.05$). The treatment containing Effective microorganisms plus manure had the highest shoot growth compared to all other treatments, about 46% growth increase compared to the control treatment (soil only) (Table-1).

Leaf number

Effective microorganisms' inoculation improved leaf production over the experimental period (Table-1). There were significant differences ($p \leq 0.05$), in leaf formation among treatments throughout the study period. Plants inoculated with EM and organic manure recorded the highest number of leaves compared to all other treatments, this was about 26% of control plants.

Leaf area

Leaf expansion occurred in all the treatments. There were significant differences ($p \leq 0.05$), in leaf area growth at the end of the experiment (Table-2). Plants inoculated with EM manure recorded the highest leaf area growth, followed by the treatments containing soil + manure about 35% of the control treatment (soil only).

Stem diameter

Stem diameter growth increased among all the treatments. There were significant differences in stem diameter growth ($p \leq 0.05$); (Table-2). Plants inoculated with EM had the highest stem diameter growth, about 45.4% of the control plants (soil only).

Leaf fresh weight: Leaf fresh weight increased in all the treatments over the experimental period, however there were significant differences ($p \leq 0.05$), among treatments. Plants inoculated with EM manure recorded the highest fresh weight, which was about 76% of the control plants (soil only).

Root fresh weight

Maximum root fresh weight was recorded in media having manure treated with EM. There were significant differences among the treatments for this parameter. Fresh weight was highest in soil inoculated with effective microorganisms.

Leaf dry weight

Plants inoculated with EM with soil and manure recorded the highest dry weight (Table-2), about 180% of the control plants (soil only). There were significant differences ($p \leq 0.05$) among all the treatments.

Root dry weight

Root dry mass was highest in soil without organic manure inoculated with effective microorganisms (Table-2). There were significant differences ($p \leq 0.05$), among the treatments.

Chlorophyll content

There was no significant change in the chlorophyll *a* and chlorophyll *b* synthesis among the treatments. However pigweeds inoculated with EM manure had relatively higher chlorophyll a and chlorophyll b content compared to all other treatments (Table-3). Chlorophyll *a* was also higher than chlorophyll *b* in all the treatments.

 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science

Table-1. shoot and leaf number increase over the 42 days experimental period.

Days since EM inoculation	**Treatment**	**Shoot height** (cm)	**Leaf number per plant**
14	EM + manure + soil	9.94a	6.2a
	Soil only	8.44b	6.0a
	Soil + manure	9.16ab	6.0a
	Soil + EM	9.06	6.0
	LSD	1.1181	0.2998
22	EM + manure + soil	14.14a	7.4a
	Soil only	12.54ab	6.4b
	Soil + manure	12.06ab	6.6ab
	Soil + EM	12.40ab	6.0a
	LSD	1.8449	0.924
30	EM manure + soil	26.97a	12.8a
	Soil only	20.980b	10.8b
	Soil + manure	23.0b	11.4b
	Soil + EM	20.7b	10.6b
	LSD	3.414	1.2542
36	EM + manure + soil	57.64a	18.6a
	Soil only	39.54c	15.6b
	Soil + manure	49.92b	18.0a
	Soil + EM	40.86c	15.0b
	LSD	6.667	1.8481
42	EM + manure + soil	61.5a	25.0a
	Soil only	43.1b	19.8b
	Soil + manure	54.98a	23.4a
	Soil + EM	44.44b	19.8b
	LSD	6.9641	1.7988

Table-2. Stem diameter, shoot and root fresh weight, shoot and root dry weight and leaf area increase over 42 days experimental period.

Treatment	**Stem diameter** (cm)	**Leaf area** (cm^2)	**Leaf fresh weight** (g)	**Root fresh weight** (g)	**Leaf dry weight** (g)	**Root dry weight** (g)
Soil + EM + organic manure	3.6a	65.55a	62.718a	10.664a	13.468a	2.25b
Soil only	2.476c	48.61d	35.628c	9.524a	4.804c	2.056b
Soil + manure	3.226b	62.092b	56.412b	7.49b	11.174b	2.424b
Soil + EM	2.662c	60.74c	19.61d	7.5b	3.566d	5.708a
LSD	0.3303	1.1931	2.0145	1.1575	0.5571	0.5445

Means followed by same letters down the column for treatments are not significantly different at $p \leq 0.05$.

Table-3. Effect of EM inoculation on leaf chlorophyll content of pigweed 42 days after inoculation.

Treatment	Chlorophyll a (mg/g fresh weight)	Chlorophyll b (mg/g fresh weight)
Soil + EM + organic manure	9.214a	5.9a
Soil only	8.522a	2.728a
Soil + organic manure	8.6a	6.130a
Soil + EM	8.94a	7.308a
LSD	3.4756	5.5839

Means followed by same letters down the columns for treatments are not significantly different at ($p \leq 0.05$).

DISCUSSIONS

The results from the study indicate that inoculation of pigweeds with effective microorganisms increased the growth of shoot height, stem diameter, leaf number, leaf area, leaf fresh and dry weights, and root fresh and dry weights. Increased shoot height stem diameter growth probably reflects allocation of resources into shoots rather than roots (Tables 1 and 2). Increase in the number of leaves and leaf area are common occurrences in plants that are provided with proper nutrition and this can increase the photosynthetic activity of the plants.

Increase in leaf area and number of leaves should result to higher rates of photosynthesis hence increased plant growth. For plants, a high rate of net carbon assimilation can result in higher biomass accumulation, favouring future growth and reproduction. The position and distribution of leaves along the shoot influences the sink strength of the plants. During early stages of leaf growth, synthesis of chlorophyll, proteins and structural compounds is high resulting in high catabolic rates to support energy needs by the plants. Inoculation of effective microorganism can increase the available nutrition for plant roots and improve photosynthesis. Singh *et al*. (2003) reported that biological seed and mucilage yield of Isabgol could be increased with application of animal manure and integrated systems due to improved soil physical and chemical properties. Accumulation of dry matter and its distribution into different plant components is an important consideration in achieving desirable economic yield from crop plants (Singh and Yadav, 1989). Chlorophyll *a* and *b* content increased in all the treatments, even though the plants inoculated with effective microorganisms had relatively higher chlorophyll contents (Table-3).

Increase in chlorophyll *a* and *b* contents of the pigweed may contribute to increased photosynthetic activity. The synthesis and degradation of the photosynthetic pigments are normally associated with the photosynthetic efficiency of the plants and their growth adaptability to different environments (Beadle, 1993). Increase in leaf chlorophyll content could in turn lead to increased protein synthesis of the plants and this could have a direct consequence on the plant growth and photosynthesis (Hendry *et al*., 1987). Nitrogen is one of the essential nutrients involved as a constituent of biomolecules such as nucleic acids, coenzymes and proteins (Sharma *et al*., 1995), any deviation in these constituents would inhibit the growth and yield of plants. Protein concentrations in plants tend to increase with fertility level of the growth medium (Grant and Bailey, 1993).

In general effective microorganisms seem to have direct impact on growth and yield of pigweeds. Previous studies have demonstrated a consistent positive response with the use of effective microorganisms in crop production and indicate the potential of this technology to reduce fertilizer use and increase the yield and quality of crops (Higa, 1991).

CONCLUSIONS

The results show that inoculation of pigweeds *(Amaranthus dubians)* with effective microorganisms can improve their growth and yields. To prevent the environmental pollution from extensive application of fertilizers, the effective microorganisms could be recommended to farmers to insure the public health and a sustainable agriculture.The data collected proves that the use of effective microrganisms can lead to higher amaranthus yield *(Amaranthus dubians)*. Further research should be done to quantify the numerous effects of EM on growth and yield of other amaranthus species. The local community should be sensitized on the use of EM to improve farming and thus help alleviate poverty; this should be done through workshops and seminars.

ACKNOWLEDGEMENTS

Mr. Peter Olewe is appreciated for his technical assistance during the laying out of the experiments in the greenhouse. We are grateful to the Department of Botany and Horticulture for financial support to carry out this study.

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