Comparative studies on the effects of soil residues of three maize herbicides on the growth, yield and proximate composition of *Amaranthus cruentus.*

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Abstract: A completely randomized design pot experiment was conducted in the screen house of the Department of Crop, Soil and Pest Management of the Federal University of Technology Akure (7°16′N, 5°12′E) Nigeria, to compare the soil residual effects of atrazine, 2,4-D and glyphosate on the growth and yield as well as leaf nutritional quality of *A. Cruentus*. Seeds of *A. Cruentus* were sown in soil samples collected from maize plots where the foregoing herbicides have been used to control weeds at the usual field recommended rates. The preliminary treatments also included a weed-free control and a weedy check. Results indicated that soils containing atrazine residues best supported the growth of amaranth as it gave the highest values in terms of all the growth parameters considered. This however did not translate to increase in yield as there were no significant differences among the treatments in the yield parameters. Results further showed that the herbicides, when applied to the field at the field recommended rates did not leave residues in the soil, which may cause phytotoxic effects to the indicator plant. Seedling development and dry matter accumulation of the indicator plant was more than 90 percent normal (when compared with those from un-treated plots). It is therefore concluded that the gestation period of 12 weeks for maize growth is long enough to degrade any of the three herbicides to be able to sustain amaranth sown to succeed maize in rotation in the rain forest vegetation zone of Nigeria.
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1. Introduction

Vegetable crops may be sown to succeed maize to 'catch' remaining moisture under rainfed multiple cropping in southwestern Nigeria (Aladesanwa and Adejoro, 2009). Pre-emergence and post-emergence applications of atrazine and 2,4-D respectively are common for weed control in maize in this region. Preplant application of glyphosate has also been observed to prevent early weed competition in maize, even beyond the critical period of weed control (CPWC) (Oral communication). Some levels of soil persistence and soil residual activity have however been reported for the foregoing herbicides.

Studies by Aladesanwa *et al.* (2001), and Aladesanwa (2007) have demonstrated clearly that celosia, okra and long fruited jute were all significantly affected by atrazine residues when these vegetables were sown 12 weeks after atrazine was applied to soils in the screen house at the usual soil application dose of 3.0 kg a.i. ha^{-1}. The soil half-life of glyphosate varies from few days to two or three months, and mostly, is smaller than one growing season; but, there are some reports of soil persistence for hundred and thousand days, and persistence of the phytotoxic activity for more than 19 weeks after the application (Heinonen-Tanski, 1989; Feng and Thompson, 1990; Weber, 1994). Although accumulation of 2,4-D is generally not a problem at recommended rates of application (1.0 kg a.i. ha–1), its indiscriminate use may lead to residue accumulation in the soil (Tejada *et al*., 1995). 2,4-D has a moderate persistence in soil with a field dissipation half-life of between 59 - 66 days.

Exclusive reliance on herbicide chemical has led to concerns about contamination of environment by the presence of herbicide residue in soil, water and plants, particularly vegetables and fruits, and this constitute a threat to human health (Chowdhury et. al., 2009**).** It is therefore important to study the soil residual effects of the three foregoing classes of herbicides on the performance and nutritional quality of *A. cruentus,* which may be sown to succeed maize in rotation.

The present study therefore aimed at comparing the effects of soil residues of atrazine, glyphosate and 2,4-D on the growth and yield as well as on leaf nutritional quality of *Amaranthus cruentus.*

2. Materials and Methods

A completely randomized pot experiment was conducted in the screen house of the department of Crop, Soil and Pest Management of the Federal University of Technology, Akure (7°16′N, 5°12′E) located in the rain forest vegetation zone of Nigeria in

2013. A preliminary field trial involving application of atrazine, glyphosate, and 2,4-D in maize at the usual recommended rates of 3.0kg a.i/ha, 2.0l/ha, and 1.0 kg a.i./ha respectively including a weed-free control, where weeding occurred once per week till harvest at 12 Weeks after Planting and a weedy control, where no weeding occurred during the experimental period, was laid out in a randomized complete block design (RCBD) with four replications per treatment. At maize harvest, soil from each plot was filled into pots measuring 4 liter each, and the pots were transferred to the screen house to test the effects of the herbicide residues on the performance of *A. cruentus.*

Seeds of *A. cruentus* were sown into the pots broadcast but were later thinned to two stands per pot after germination. Watering was done at two days interval, no fertilizer was applied, and emerged weeds were hand-pulled from the pots.

Growth parameter (plant height and number of leaves per plant) were taken on weekly basis beginning from the fourth week up to 8 weeks after planting. plant height was measured using a tape in centimeters. Average leaf number was determined by counting the total number of leaves of two plant stand in each pot and recording the average. At harvest, yield parameters as well as leaf area were determined.leaf proximate analysis was also carried out at *A.cruentus* harvest. Proximate analysis involving the moisture content, protein, fat, Ash, Carbohydrate, and crude fiber were determined according to the standard method of AOAC (1990).

Moisture Content Determination: 2g of each samples were weighed separately into previously weighed petri-dishes. The samples and the dishes were placed in a thermostatically controlled oven at a temperature of 1050^0c for 3 hours. Drying was continued until a constant weight was obtained as

$$\%moisture = \frac{weight\ loss\ \times 100}{weight\ of\ sample}$$

Fat Content Determination: Soxhlet extractor was fixed with a reflux condenser together with a small flask, which has been previously dried in the oven.2g of each sample were weighed (W2). Into a filter paper already weighed (W1). 500ml fat free round bottom flask was filled with petroleum ether up to ¾ of the 500ml flask. The Soxhlet extractor was fixed up with a reflux condenser. Heat source was adjusted so that the solvent boils gently, it was allowed to siphon for four hours. The condenser was detached and the filter paper was removed. The filter paper was placed in a beaker in an oven at 50^0c for 30 minutes to constant weight with sample and then cooled in a desiccator and weighted (W3). Percentage moisture content was then determined as $\%Moisture = \frac{\%\ Fat=W2-W3\ \times 100}{weight\ of\ sample}$

Protein Content Determination: The first step involved is the digestion in which about 0.5g of each sample were digested separately with concentrated H_2SO_4 (10ml) together with 2 tablets of mercury catalyst (or 2 tablets of either selenium or copper). The sample was digested by heating in an electro-thermal heater in a fume cupboard until a clear solution was obtained. The liquid was cooled after which the solution was diluted with water to $100cm^3$ of which $10cm^3$ was transferred into the Kjeldahl distillation apparatus.

The next step was distillation stage, this involves stem distillation of the cooled, diluted, digested sample to which 40% NaOH solution was added to make it alkaline, 3 drops of screened methyl red indicator was added to the receiving flask containing 10ml of 2% boric acid solution to produce a pink colour solution. The cloudy nature of the sample solution after the addition of 40% NaOH indicated that NaOH was in excess. The solution in the receiver was removed and titrated with standard HCl to a deep blue colour indicating the presence of ammonia. The volume used in titration was estimated and recorded as percentage nitrogen.

$$\%Nitrogen = \frac{volume\ of\ acid\ (Hcl) used\ 0.0014gx100x100}{weight\ of\ sample\ x5}$$

Ash Content Determination: The ash content of each sample was determined based on the residue after the burning of organic component. A certain amount of samples were burnt in a muffle furnace at 500^0c for seven hours. The residue was then cooled, weighed and recorded are the percentage ash content was calculated as

$$\%ASH = \frac{w3 - w1\ x100\%}{w2 - 1}$$

Crude Fibre Determination: Ether extracted residue was transferred into 500ml conical flask. The original was W1 200ml of boiling 1.25% H_2SO_4 was added and brought to boil within one minute and it was allowed to boil gently for 30 minutes cooling finger was exacted to maintain constant volume. It was then filtered through a filter paper by suction using buncher funnel and rinsed well with hot water and the material was separated back into the flask with the aid of a spatula. 200ml of boiling 1.25% NaOH was added and brought to boil within one minutes and it was then boiled gently for 30 minutes. By fingering it was filter through poplin cloth and washed with hot water. Then it was rinsed with hot

distilled water and once with 10% HCl, four times with hot water again, twice with methylated spirit and 3 minutes with petroleum ether, ethanol could be used as a substitute for methylated spirit. The empty crucible was weighed and preheated in oven also cooled in a dedicator W2. The residue was served into already weighed crucible and oven dried at 105^0c, then cooled in a desiccator and weighed W3. It was placed in a muffle furnace at about 200^0c for 30minutes. It was removed from desiccators and allowed to cool to room temperature and the weight was determined.

Analysis of Data: Analysis data collected were subjected to Analysis of Variance (ANOVA) and means separation was done using the Duncan Multiple Range Test (DMRT).

3. Results

Results of the effects of the different treatments on the growth and yield as well as nutritional quality of *A.cruentus* are presented on the tables below. The effects of the different weeding treatments on the plant height of *A.cruentus* are presented in table 1. At 4 WAP atrazine treatment produced significantly taller maize plants compare to the weedy check, while all other weeding treatments did not influence maize heights significantly ($P<0.05$) compared to the unweeded control. The same trend of influence was observed for the weeding treatments on plant height in subsequent weeks. However, at maize harvest, glyphosate treatment produced the shortest stands of Amaranth reducing plant height significantly relative to the atrazine and 2,4-D treatments. The tallest stands of maize were obtained in soil samples previously treated with atrazine, where significantly higher values were recorded compared to all the other treatments.

Results of the effects of residual treatments of the three herbicides on the average number of leaves and leaf area of *A. cruentus* are presented in table 2. Significant differences in average number of leaves were not recorded among the treatments from the 4^{th} week after planting up to 6WAP. However, the atrazine treatment recorded the highest number of leaves at the time of harvest, and figure obtained from this treatment (41.83) was significantly higher than those from the glyphosate treatment (20.00). None of the herbicide treatments significantly affected the leaf area of *A. cruentus* compared to the controls. The broadest leaves were however obtained from plants grown on soil previously treated with atrazine.

Table 1: Effects of weeding treatments on the plant height (cm) of *A. cruentus*

Treatment	Weeks after planting				
	4	5	6	7	8
GLY	12.67ab	23.83b	28.1667b	32.33b	35.00c
ATR	21.1667a	34.83a	45.1667a	47.66a	67.0a
2, 4- D	14.83ab	28.83ab	36.83ab	41.166ab	49.33b
WEED FREE	13.00ab	21.50b	31.50b	38.66ab	45.66bc
WEEDY CHECK	9.1667b	19.33b	30.83b	36.33ab	47.166bc

Means with the same letter in same column are not significantly different from one another

Table 2; Effects of weeding treatments on average leaf number and leaf area (cm^2) of *A. cruentus*

Treatment	**Weeks after planting**					
	4	**5**	**6**	**7**	**8**	**Leaf area**
GLY	11.00a	13.33 a	17.50 a	14.50b	20.00b	8.43b
ATR	14.16 a	17.33a	18.16a	21.00a	41.83a	11.96a
2, 4- D	13.16 a	15.66a	19.16a	23.50a	26.16ab	11.07ab
WEED FREE	11.66 a	15.33a	16.16a	23.16a	34.33ab	11.03ab
WEEDY CHECK	11.17 a	15.83a	18.50a	21.00a	26.16ab	10.47ab

Means with the same letter in same column are not significantly different from one another

Table 3 shows the effects of the different weeding treatments on the yield parameters considered. None of the treatments significantly affected edible yield, marketable yield and total biomass of *A. cruentus* at harvest, and the influences of the different weeding treatments are also not consistent on the yield attributing parameters. While the highest edible and total biomass were recorded against the weed-free treatment, weed control with atrazine resulted in the highest marketable yield of the test vegetable.

Table 3: Effects of weeding treatments on the yield of *A. cruentus*

Treatment	Edible yield (g/m^2)	Marketable yield (g/m^2)	Total biomass (g/m^2)
GLY	9.33a	19.83a	21.33a
ATR	10.66a	25.66a	27.33a
2, 4- D	12.50a	24.66a	26.50a
WEED FREE	13.83a	23.66a	28.66a
WEEDY CHECK	8.50a	18.00a	19.33a

Means with the same letter in same column are not significantly different from one another

Results indicating the effects of the various weeding treatments on leaf proximate composition are presented in table 4. The weed free treatment produced *A. cruentus* with the highest percentage of leaf crude fiber, ash and protein. The weedy check has the highest percentage of moisture content while percentage fat is highest in the atrazine treatment. Among the herbicide treatments, 2,4-D recorded the highest values in crude fibre (14.12%), Moisture content (7.98%) and ash content (23.98%), while glyphosate and atrazine gave the highest percentages of protein (17.08) and fat (5.16) respectively.

Table 4: Effects of weeding treatments on leaf proximate composition of *A. cruentus*

Treatment	Crude fiber (%)	Moisture content (%)	Ash (%)	Protein (%)	Fat (%)
GLY	13.56bc	6.88c	13.56c	17.08c	.0.78c
ATR	11.00d	6.07c	19.39b	16.06d	5.16a
2, 4- D	14.12b	7.98b	23.98a	13.14e	0.66cd
WEED FREE	39.17a	6.68c	24.17a	22.22a	2.43b
WEEDY CHECK	11.64cd	9.11a	23.41a	19.96b	0.50d

Means with the same letter in same column are not significantly different from one another

4. Discussions

All the treatments increased plant height over the weedy control at 4WAP, but this increase was only significant ($P<0.05$) in treatments with atrazine residues. This could be explained by the fact that glyphosate, atrazine, 2,4-D all undergo degradation by soil microorganisms **(**Radosevich, 1994) and the significant increase caused by atrazine might be as a result of NH_4-N made available as a degradation product of atrazine (Sene *et al.*, 2010), which presumably increased microbial activity and N intake by the test crop. This by extension indicates the presence of microbial strains capable of completely degrading atrazine within the 12 weeks of maize growth in the preliminary experiment. It further suggested that the presence of the residues may enhance the activities of the soil microbes. This also explains the highest plant height recorded in atrazine treatment at 8 weeks after planting. The fact that significant differences were not recorded among the treatments the yield parameters considered was an indication that all the chemicals applied were no more phytotoxic to vegetables at 12 weeks after application. Significant differences occur among the various treatments regarding the leaf proximate composition of *A. cruentus* but no consistent trend was maintained among the parameters. In the present study, the different herbicide treatments did not consistently affect seedling growth of *A. cruentus* in relation to plant height, leaf area and accumulation of dry matter, thus either indicating absence of residue, or the residues were un-available to the plants. Streibig (1984 & 1988) indicated that when indicators of plant growth were plotted against logarithm of herbicide dose, they were common to find a symmetrical sigmoid relationship. Since the plant growth parameters among different atrazine doses did not show consistent significant differences, a symmetrical sigmoid curve of the dose response curve would not have been obtained. Pestemer *et al*., (1980) proposed that adverse effect of herbicide residues in soil could be determined with plant response, and went ahead to classify indicator of plant responses to residual herbicides into the activity categories of stimulation (100% safe), no response (90-100% safe), slight damage (70-90%, slight risk), moderate damage (50-70%, significant damage) and severe damage (0-50%, crop failure). Thus, treatment with the herbicides at the usual recommended rates gave an indicator response showing the treatments were safe (>90% response) and did not cause adverse effect to most of the growth, yield and leaf nutrient quality components of the plants compared to the untreated plots. Aladesanwa and Adejoro (2009) in a related field experiment with *Amaranthus cruentus* as test crop for atrazine earlier noted that amaranth is not quite sensitive to atrazine residue from maize cropping and may be sown to succeed maize in rotation without suffering a concomitant reduction in crop growth and yield under rainfed multiple cropping. The present

study has also clearly indicated that glyphosate and 2, 4-D can also be applied to control weed in a short season crops like maize without leaving residues in soils to adversely affect subsequent crops in a rotational cropping system.

5. Conclusions

Atrazine, glyphosate and 2,4-D when applied to the field at the usual field recommended rates did not leave residues in the soil, which may cause phytotoxic effects to the indicator plant, amaranth. Seedling development and dry matter accumulation of the indicator plant was more than 90 percent normal (when compared with those from un-treated plots). The gestation period of 12 weeks for maize growth is therefore long enough to degrade these herbicides, to be able to sustain amaranth sown to succeed maize in rotation in the rain forest vegetation zone of Nigeria. The conflict between this result and those of previous studies must have been clearly due to the discrepancies between the field environment and the homogeneity of the screen house environment.

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