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Effects Of 2,4-Dichlorophenoxyacetic Acid (2,4-D) Herbicide On The Growth, Yield And Carbohydrate Content Of Maize (*Zea mays*)

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Abstract
A field experiment on the effects of 2,4-dichlorophenoxyacetic acid herbicide on the growth, yield and carbohydrate content of maize (Zea mays) was carried out at Federal College of Forestry, Jos. The experiment consisted of five treatments, in which the control plot was weeded using hoe. Other treatments are T_1 (4 ml/L), T_2 (8 ml/L), T_3 (16 ml/L) and T_4 (32 ml/L) of 2,4-D herbicide respectively applied to $4m^2$ experimental plot for weed control. The experimental design consisted of Randomized Complete Block Design and data was taken on the germination number, leaf count, plant height, number of tassels, yield and starch concentration of maize grains. Statistical package for Social Sciences (SPSS), version 23 was used to carry out data analysis and Tukey's test was used to separate the means where significant differences occurred. The results showed that significant differences occurred ($p \leq 0.05$) on the germination number, leaf count, plant height, number of tassels, yield and the carbohydrate content of the maize. Treatments T_3 and T_4 however gave conspicuous lower values for each of the parameters assessed. Conclusively, the application of 2,4-D should be done at doses not above 8 ml/L for the control of weeds in maize fields and to avert toxicity effects on the resulting yield.

Keywords: *Maize, 2,4-dichlorophenoxyacetic acid (2,4-D), growth, yield, starch concentration.*

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I. Introduction

Maize is one of the most significant cereal crops of the world. In most of the developing countries, maize contributes to the food security after rice and wheat in term of areas harvested and in terms of annual production, maize is ranked first. (1) Maize occurs as the most important cereal crops in sub-Sahara Africa and occupies about 33 million ha of land yearly in term of production. (2) Maize is not only used as human food and animal feed but it is also widely used for corn oil production, corn starch industry, baby corns etc. (3) Maize is the world's most widely grown cereal as it is grown in a range of agro-ecological environments and more maize is produced annually than any other grain. (4)

Worldwide production of maize is 1,048,610,000 metric tons in the year 2014, with the largest producer, the United States, producing 377.5 million metric tonnes. (5,6) Africa produces 6.5% and the second largest African producer is Nigeria which produces 8,694,900 tonnes in 2014. (7) Africa import 28% of the required maize form countries outside the continent. Most maize production in Africa is rain fed. Irregular rainfall can trigger famine during drought. (8) Maize production in Nigeria formerly occur around the forest zone but the cultivation of maize now extends to the savannah region of the country where over 70% of the production occurs. (9)

Maize is a cereal crop plant which can be consumed in so many ways such as raw cooking, roasting, frying, grinding, pounding or crushing in order to prepare various food items like pap, tuwo, gwate, donkunu and a host of others. (10) These food items are commonly consumed by various tribes in all parts of Nigeria. Some of these tribes include Yorubas, Hausas, Ibos, Ibiras, Ishas, Binis, Efiks, Plateau and Yalas etc. Maize provide abundant nutritional benefits to all age groups. Its high amount of carbohydrate, which the highest percentage makes it a good energy food. The carbohydrate component of the food is readily hydrolysable and this makes it to be suitable for consumption in the morning before carrying out individual's daily activities.

Considering factors responsible for low yield, weed infestation is of prime importance. (11) Weeds compete with crops for space, light, moisture, nutrients and carbon dioxide [1], which reduces not only the yield, grain quality (12) and hinder harvest operations but also increase the cost of production. (13) Excessive growth of weeds in maize field leads to 66 % to 80 % reduction in crop yield. (14,15) Herbicides have for many years showed promising effects in the control of weeds on the farm lands and this has brought about a great relieve to farmers. Some of these herbicides are weed specific as they are effective against grasses while others target broad leaved weeds. 2,4-dichlorophenoxyacetic acid (2,4-D) herbicide is an example of the selective herbicide which is widely used for the control of broad leaf weeds on the farm, gardens, roadways, parks etc. (16) It is a pre-emergence as well as a post emergence herbicide which is highly effective for the control of broad leaves and not grasses. Its ease of solubility in water and other solvents results in fast root and leaf penetration. 2,4-dichlorophenoxyacetic acid also regulates plant growth by mimicking natural auxin through its ability to promote cell division and elongation. (17)

Herbicide usage for the cultivation of crops have been greatly discouraged due to their numerous side effects to plants, environmental pollution and their side effects on the health of animals and man. 2,4-dichlorophenoxyacetic acid (2,4-D) occur in both acidic and salt states and their ester formulations have been reported to be toxic to fish and aquatic invertebrates. (16) Although, 2,4-dichlorophenoxyacetic acid (2,4-D) have been said to be relatively low in term of its toxicicty, its ability to cause eye irritation effects have been reported.

II. Materials and Methods

Study Area

This research was carried out at the experimental field of Federal College of Forestry Jos. The college is located in Jos North Local Government Area of Plateau State, between latitude 7^oN and 11^oN and longitude 70^o and 25^o East with an elevation of 1,200m above sea level. It has a climate generally of tropical humid with an annual rainfall of about 146 – 148 m and approximate annual temperature of 10^o – 32^oc minimum and maximum. It contains sandy-loam soil light brown in color, the soil is well drained and aerated. Jos North Local Government has a projected population of 500,410. (18)

Source of Planting Materials

The maize seed was obtained from P.A.D.P, Plateau State. The N.P.K (30:30:30) fertilizer used was gotten from the market, while other materials used such as the farm implements and tools were obtained from the Crop Production Technology department, Federal College of Forestry, Jos.

Method

The herbicide was properly mixed based on the requirements for each treatment. Each plot was marked out after which the maize plant was planted. The 2,4-D herbicide was applied to each of the plots after land preparation and as soon as weeds appeared while the control plots was weeded manually using hoe. The herbicide was applied three times during the experimental period.

Determination of Carbohydrate Content

Determination of the carbohydrate content of the maize grain was done with the method described by (19) with some modifications. 0.1g of the pulverized sample was weighed into a volumetric flask after which 1 mL of distilled water and 1.3 mL of perchloric acid were added. The content was mixed properly by shaking for 30 minutes and the flask made up to 25 mL with distilled water. The solution was filtered through Whatmann No.1 filter paper. 1 mL of the filtrate was transferred into a test tube and was diluted to volume with distilled water. 1 mL of the solution was pipetted into another test tube and 5 mL of anthrone reagent was added. The blank contained 1 mL of distilled water added to 6 mL of anthrone reagent. A standard glucose solution was equally prepared containing 0.1 mL glucose with 0.9 mL distilled water and 6 mL anthrone reagent. The samples were read at 630 nm wavelength using spectrophotometer. The absorbance of the standard glucose was calculated using the formula; % carbohydrate = 25 x absorbance of sample/ absorbance of standard glucose.

Agronomic Practices

The experimental land was properly cleared using hoe, cutlass after which rake was used to remove the grasses, stumps and debris. Beds of 2 m x 2 m were made and the maize seeds were planted at a distance of 25 cm between plant stands and 75 cm between rows. N.P.K (20:10:10) fertilizer was applied at 3 weeks after germination and the second dose was applied just before flowering. The plots were weeded by using a hoe for the control treatment alone, while the experimental treatments were weeded by the application of various concentrations of 2,4-dichlorophenoxyacetic acid. The first application of the herbicide was done after land clearing, while the next applications were at the time of the next emergence of weeds. At the time of maturity, the maize was harvested by removing the cobs after which the grains were removed and weighed.

Experimental Design and Treatments: The experiment consisted of Randomized Complete Block Design, involving five treatments and each of the treatments were replicated four (4) times;

i. T_0 = manual weeding with the use of hoe
ii. T_1 = 4 mL of 2,4-D mixed with 996 mL of water per plot
iii. T_2 = 8 mL of 2,4-D mixed with 992 mL of water per plot
iv. T_3 = 16 mL of 2,4-D mixed with 984 mL of water per plot
v. T_4 = 32 mL of 2,4-D mixed with 972 mL of water per plot

Parameters Collected

i. Number of Leaves: The number of leaves was counted for the selected plant samples at two weeks after planting (2 WAP) 4 WAT, 6 WAT, 8 WAT and 10 WAP respectively.

ii. Plant Height: The height of the plants were measured from the base of the plant to the top-most part with the use of a meter rule. This was done for all the treatments at 2 WAP, 4 WAT, 6 WAT, 8 WAT and 10 WAP and the average was taken and used for further analysis.

iii. Number of Tassels: The number of tassels for all the treatments were counted from the time of tasseling and the mean values were recorded for further analysis.

iv. Number of Grains: Samples were picked from each of the treatments and the average number of grains for each treatment was recorded for further analysis.

v. **Yield:** The weight of the yield for each of the treatments was taken by placing the yield on a graduated weighing balance. The weight of the treatments were compared against that of the control.

vi. **Starch Content:** the yield obtained from each of the treatments were properly grounded after which they were extracted petroleum ether. The extracts were replicated three times and each of them was subjected to readings to measure the starch concentration. The average was taken and then analyzed to quantify the amount of starch in the grains.

Statistical Analysis: All data collected were subjected to one way analysis of variance (ANOVA) at 5% level of significance using statistical package for social students (SPSS) Version 23. Duncan Multiple Range Test (DMRT) was used to separate the means where differences occur in the means.

III. Results and Discussions

Germination Percentage

The result of the germination number showed the differences that occurred in the treatments due to the different levels of the herbicide applied. The result showed significant differences across all the treatments and at the different days in consideration. On day 1, T_0 gave the highest mean germination number (20.25) and it was observed to be statistically similar to T_1 (17.25), T_2 (15.25 and T_3 (14.50) respectively. Treatment T_0 was however seen to be significantly different from T_4 (9.75), with the latter being statistically similar to T_1, T_2 and T_3 respectively. Similar results were observed on day 2 as well as day 3 but not on day 4 where there was no significant difference between all the treatments.

Table 1: Effects of 2,4-D Herbicide on the Germination Number of Maize (*Zea mays*)

Groups	Germination Number Day 1	Germination Number Day 2	Germination Number Day 3	Germination Number Day 4
T_0	20.25^{a}	28.50^{a}	39.00^{a}	40.50
T_1	17.25^{ab}	26.75^{ab}	36.75^{ab}	38.25
T_2	15.25^{ab}	26.00^{ab}	36.00^{ab}	41.25
T_3	14.50^{ab}	22.50^{ab}	35.50^{ab}	39.50
T_4	9.75^{b}	17.50^{b}	31.00^{b}	39.50
SEM	3.66	4.61	2.79	2.22
L.S	*	*	*	N.S

Means in the same column having the same alphabets are not significantly different

Leaf Count

The effect of 2,4-D herbicide on the number of leaves is as displayed in table 6. The result showed significant differences between the groups at 6 WAP, 8 WAP and 10 WAP respectively. However significant difference was not observed at the initial stage of the experiment, which are at 2 WAP and 4 WAP. Treatments T_0 (7.2) and T_1 (7.35) gave highest mean value at 2 WAP, while treatment T_1 (7.35) produced the highest mean value at 4 WAP which was not significantly different from the other groups. The highest mean value was observed to be from T_0 (11.28) at 8 WAP and 10 WAP (13.88) respectively.

Table 2: Effects of 2,4-D Herbicide on the Leaf Count of Maize (*Zea mays*)

Groups	L.C @2WAP	L.C @4WAP	L.C @6WAP	L.C @8WAP	L.C @10WAP
T_0	4.75	7.20	8.70^a	11.28^a	13.88^a
T_1	4.75	7.35	7.03^b	9.73^b	10.75^b
T_2	4.53	6.73	6.83^b	10.08^b	11.08^b
T_3	4.65	6.83	7.05^b	9.78^b	11.48^b
T_4	4.40	7.10	7.33^b	10.08^b	9.70^c
SEM	0.27	0.46	0.25	0.36	0.45
L.S	N.S	N.S	*	*	*

Means in the same column having the same alphabets are not significantly different

Plant Height

The results displayed in table 5 showed the effects of 2,4-D herbicide on the height of maize. The results showed that there was no significant difference between the treatments at 2 WAP. Significant differences were observed at 4 WAP, 6 WAP, 8 WAP and 10 WAP. The highest mean values were observed in T_0 at 2 WAP (17.20), 4 WAP (34.03), 8 WAP (102.08) and 10 WAP (113.4) respectively. Treatment T_2 produced the highest mean value (50.43) at 6 WAP, while the lowest mean value was observed in treatment T_4. The effect of the herbicide was more obvious in the treatment with the highest concentration of the herbicide, where it gave the lowest mean values at 2 WAP (16.20), 6 WAP (40.80) and 10 WAP (90.93) respectively.

Table 3: Effects of 2,4-D Herbicide on the Plant Height of Maize (*Zea mays*)

Groups	P.H @ 2WAP	P.H @4WAP	P.H @6WAP	P.H @8WAP	P.H @10WAP
T_0	17.20	34.03^a	49.01^a	102.08^a	113.40^a
T_1	15.38	27.65^b	48.08^a	81.13^c	100.55^b
T_2	17.18	28.53^b	50.43^a	87.55^{bc}	94.10^c
T_3	17.00	29.93^b	46.63^a	89.70^b	94.58^c
T_4	16.20	28.60^b	40.80^b	90.65^b	90.93^c
SEM	0.89	1.22	1.23	2.52	1.24
L.S	N.S	*	*	*	*

Means in the same column having the same alphabets are not significantly different

Number of Tassels

The effect of 2,4-D herbicide on number of tassels showed that differences occurred among the treatments starting from day 2 to day 8. Significant differences were observed to occur from day 2 to day 8 on number of tassels. Treatment T_0 gave the highest mean value of 5.75, 10.75, 12.25 and 28.75 on day 2, day 4, day 6 and day 8 respectively. It was observed that between the experimental treatments, T_2 produced means that stood out from the other treatments.

Table 4: Effects of 2,4-D Herbicide on the Number of Tassels of Maize (*Zea mays*)

Groups	Tassels (Day 2)	(Tassels Day4)	(Tassels Day 6)	(Tassel Day8)
T_0	5.75^a	10.75^a	12.25^a	28.75^a
T_1	4.75^{ab}	6.00^{ab}	7.00^{ab}	14.25^b
T_2	4.75^{ab}	7.25^{ab}	9.50^{ab}	19.50^b
T_3	2.25^b	3.75^b	5.25^c	12.75^b
T_4	1.50^b	2.50^b	3.50^c	12.75^b
SEM	1.83	2.48	2.72	3.93
L.S	*	*	*	*

Means in the same column having the same alphabets are not significantly different

Yield Weight

The mean weight of the maize yield explained the effects of the herbicide on the yield of maize (Table 7). Significant differences were observed to occur between the treatments. Treatment T_0 was significantly different from T_3 and T_4, while T_1 and T_2 are statistically similar to T_0. Treatments T_1 and T_2 were also observed to be statistically similar to T_3 and T_4. The highest mean value occurred in T_0 (0.51), while T_1 produced the second highest mean value (0.34). Treatment T_4 gave the lowest mean value for the yield obtained.

Table 5: Effects of 2,4-D Herbicide on the Yield Weight of Maize (*Zea mays*)

Groups	Mean Weight (Kg)
T_0	0.51^{a}
T_1	0.34^{ab}
T_2	0.25^{ab}
T_3	0.21^{b}
T_4	0.14^{b}
SEM	0.10
LSD	*

Means in the same column having the same alphabets are not significantly different

Grain Starch Concentration

The total starch concentration of maize as affected by the herbicide usage is as displayed in figure 2. The result showed that significant differences occurred across the treatments, and these differences occurred in a dose dependent manner. Treatment T_0 was significantly different from T_3 and T_4, while T_0 was not seen to be significantly different from T_1. Treatments T_1 and T_2 are however statistically similar to each other, and likewise treatments T_2 and T_3, as well as treatments T_3 and T_4 were all statistically similar to one another.

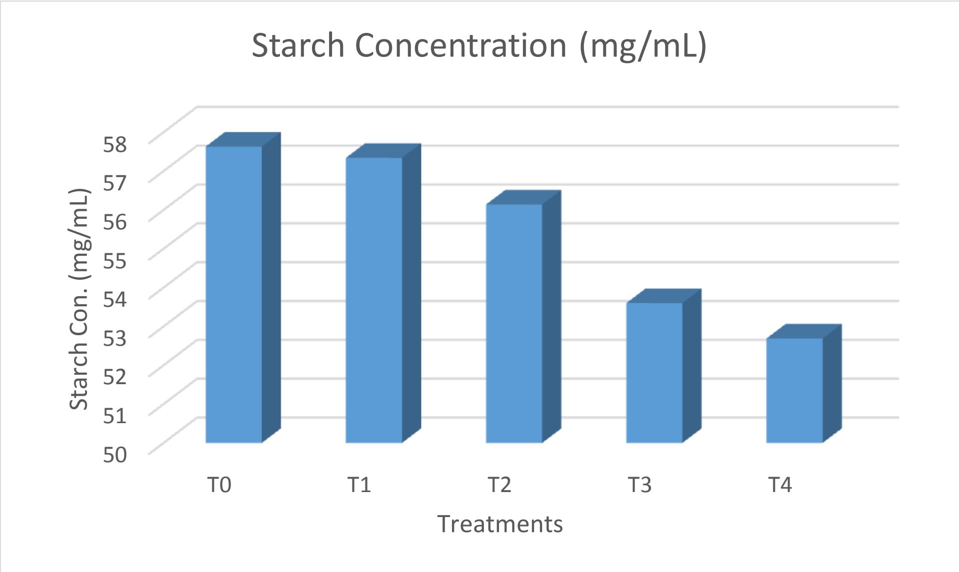

Fig 1: Effect of 2,4-D Herbicide on Carbohydrate Concentration of Maize (*Zea mays*)

IV. Discussions

The significance differences observed on day 1, day 2 and day 3 for the seed germination could be due to the effect of the herbicide. This could be as a result of the effect of the herbicide on plumule and radicle elongation, thereby causing delay in the germination of the seeds. There could be an increase in the soil acidity which led to a gradual cell death, and an eventual reduction in the germination number of the treatments with higher 2,4-D herbicide. The herbicide effect could however not cause a significant effect on day 4 and this could be due to the rapid degradation rate of 2,4-D in the soil. This result is similar to the work published by (20) in which glyphosate herbicide was reported to cause decrease in the germination percentage in *Hibiscus cannabinus*.

The significant differences observed at higher doses of the herbicide and at 6 WAP, 8 WAP and 10 WAP must have been due to the effect of the herbicide. 2,4-D herbicide has been reported to interfere with a number of cellular processes in the leaves of crop plants and such processes include guard cells, stoma, palisade parenchyma and spongy mesophyll tissues. (21) 2,4-D has also been reported to induce reduction in leaf biomass formation due to the degradation of chlorophyll pigments and nDNA in mungbean. (22) This result is in line with the published research article by (23) in which a significant reduction in leaf count of groundnut was reported after the application of glyphosate herbicide at higher doses.

The differences observed in the plant height across the treatments at 2 WAP, 4 WAP, 6 WAP, 8 WAP and 10 WAP could be due to the 2,4-D herbicide applied. 2,4-D acts as a plant growth promoter when applied at lower doses. It does this by causing cell elongation in the plants. However, when applied at higher doses, it could be detrimental to the plants by causing cell growth inhibition through alteration of cell wall plasticity and by influencing protein synthesis. These could lead to the crops having stem curling, leaf withering and hence, cell death in the crop plant. (17) The obtained result is similar to that of (24) that stated that sunflower height was affected by the application of fluorochloridone when combined with s-metolachor herbibicide.

2,4-D as an herbicide is reported to act as a growth enhancer if applied at quite lower doses, but it could also inhibit the growth of crops when the concentration is much. This can be used to explain the variations observed in the maize plant at the tasseling stage where by appreciable number of tassels were recorded at T_2 which represent lower doses of the herbicide, compared to the lower number of tassels recorded at T_4 respectively. This result can be likened to the observations reported by (25,26) which stated that flowering was greatly induced by the application of 2,4-D herbicide in sweet potato when applied at 100 ppm, but morphological and physiological changes occurred that led to the reduction in flowering at 300 ppm and 500 ppm respectively.

The significant reduction in the yield can be attributed to the various effects recorded in the growth parameters of the maize plant. The higher yield obtained in T_0, T_1 and T_2 could be due to improved germination, significant increased plant height, higher leaf count and better tasseling recorded as discussed earlier. This result is similar to that of (27) in which higher grain yield was recorded in *Zea mays* after the application of herbicide. The result is however contrary to that of (28) in which the plots with weeds controlled by the use of herbicide gave higher maize grain yield compared to those in which the weeds were controlled using manual methods.

The significant reduction recorded in the carbohydrate content can be attributed to the effect of the 2,4-D herbicide applied. The herbicide could have interfere with the mechanism of starch synthesis in the crop plant. The herbicide must have demonstrated its action on the starch synthase enzyme, which is the enzyme responsible for the synthesis of starch. The obtained results was similar to that of (29) who reported that pyrimethanil and fludioxonil fungicides brought about reductions in the soluble carbohydrate and starch content of grapevine.

V. Conclusion

Conclusively, the use of 2,4-dichlorophenoxyacetic acid (2,4-D) can cause some growth retardations in maize plant when applied at a higher concentration. These effects could be much more pronounced as the concentration of the herbicide increases. The effects of the herbicide might not be noticeable at the initial stage of the application, the herbicide might need to be adsorbed into the plant and then elicit its deleterious effects.

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