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# THE EFFECTS OF GENETICALLY MODIFIED CROPS ON PHYSICOCHEMICAL PROPERTIES OF SOIL

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**ABSTRACT:** Genetically modified crops are already phenomenally successful and are grown worldwide in more than 18 countries on more than 67 million hectares which increases by more than 10% annually. Nigeria, in October 2018 joined the many other countries by approving *Bacillus thuringiensis* (*Bt*) cotton and maize, therefore, there was the need to carryout environmental risk assessment studies. A total of fifteen (15) four litter (4L) octagonal ceramic pots were filled with four kilograms (4Kg) of soil and placed on bench in two rows of ten pots each and a third row of five pots. First row pots were used to plant GM cotton seeds, while the second row pots were used for non GM cotton seeds and a third row of five pots served as control, all in the screen house. The GM cotton seeds were collected from National Biosafety Management Agency, Abuja while the non GM cotton seeds were collected from seed bank of Tissue Culture Unit of NABDA. The pH for initial, GM cotton, non GM cotton and control soil were 6.28, 6.26, 7.25, 8.26 and the percentage moisture was 0.63, 0.78, 0.89 and 0.82 respectively while the percentage Nitrogen was observed to be 17.79, 1.14, 1.10 and 0.56 respectively. Other parameters include, varying concentrations of Potassium (0.46, 1,284.47, 1,785.48, 1,252.83 mg/kg) and Phosphorus (18.76, 17.76, 16.87, 15.23 mg/kg) were recorded for the four treatments respectively. The soil consisted mainly of silt (32.09 to 34.66%) and clay (58.89 to 60.23%) while the sand content ranged from 6.11% to 7.68% reflecting the soil texture as silty – clay. The results were then tested with ANOVA at less than 0.05 P-value and no pair was found to be significant as well. The results suggest that, the GM crops have no significant effect on bacterial ecology of the soil and in turn no direct or indirect effects on human health.

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KEY WORDS: *Bacillus thuringiensis (Bt)*, Transgenic crop, Physiochemical parameters, Cotton plant.

## 1.0 INTRODUCTION

### 1.1 Background to the Study

Genetically modified (GM) crops/plants are already successful phenomenally; Eighteen countries worldwide grow GM crops on more than 67 million hectares (ha) and this amount increases by more than 10% annually (James, 2010; James, 2011; Gruissem, 2015). This remarkable growth occurred over 2 decades ago, when the Flavr Savr tomato being the first transgenic crop became available to farmers (Raman, 2017; Kamle *et al.,* 2017), seven such crops are being grown currently – Cotton (Bromoxynil resistance), Canola (increased oil production), Maize, Papaya, potato, soya bean (Glyphosate resistance) and squash, with the world most bioengineered hectare being cotton (7 million ha), maize (10 million ha) and soyabean (33 million ha) (Bawa and Anilakumar, 2013; Raman, 2017). In 2009, more than 134 million hectares were cultivated with biotech crops in 25 different countries all over the world with prediction of further increase in the near future, especially in developing countries such as those in Africa (James, 2011).

Since they were commercialized, GM crops have been beneficial both environmentally and economically thereby increasing global food crop yield by >370 million tonnes over a relatively small acreage

area (Raman, 2017).Two agronomic traits accounts for virtually all planted hectares - resistance to herbicides and resistance to insect pests (Huang *et al*, 2003; James, 2010; Brookes and Barfoot, 2015; Kamle *et al.,* 2017).Currently, the GM crop pipeline has expanded to cover other fruits, vegetables and cereals such as lettuce, strawberries, eggplant, sugarcane, rice, wheat, carrot, etc, with planned uses to increase bioproduction of vaccine, animal feed nutrients as well as present salinity and drought resistant traits for plant growth in unfavourable environmental and climatic conditions (Bawa and Anilakumar, 2013; Zhang *et al.,* 2016; Raman, 2017). GM crops are modified using recombinant DNA technology in three (3) different ways, which are; transgenic, cisgenic and intragenic. Transgenic which involves the insertion of foreign DNA from unrelated species or genus as seen in cotton, Cisgenic which involves insertion of one or more gene from related species or crossable donor as seen in potato and Intragenic which involves the use of genetic elements from other plant's sexually compatible gene pool which are then combined with promoters and terminators (Kamle *et al.,* 2017).

Three categories of GM crops can then be distinguished and they include; First generation GM technologies which involves the development of microbial (fungal, bacterial and virus) resistance in root and tuber crops as well as some cereals, also, abiotic stress tolerance such as heat, salt and drought is intensified. Second generation GM technologies which involves the improvement in product qualities for industrial and nutritional purposes and can be seen in maize with high amylose enhanced with essential amino acids, minerals and vitamins, oil seeds with improved fatty acid profiles. Third generation GM crops which involves molecular farming whereby the crops are used in the production of either industrial materials such as biodegradable plastics and enzymes or pharmaceuticals such as monoclonal antibodies and vaccines (Giannakas and Yiannaka, 2008; Hefferon, 2015; Jan and Shrivastava, 2017; Gatew and Mengistu, 2019; Nalluri and Karri, 2020). Genetically modified crops made from them has turned the agricultural sector of the world around by finding solutions to conventional breeding problems as well as its important role in meeting the growing population's food demand (Doebley *et al.,* 2016; Zhang *et al.,* 2016; Jan and Shrivastava, 2017; Nalluri and Karri, 2020).

GM crops are expected to be widely adopted for their great potential in agriculture (Huang *et al*., 2003; Mocali, 2010), but since the release of GM crops there has been a great controversy over the unexpected potential effects on the environment and human health(Bownas, 2008; Mocali, 2010). After all, with GM technology, traits can be obtained that were previously not present in crops, these new traits may have direct or indirect effect on the environment due to different methods of cultivating the new crops (Sanvido *et al*., 2012). Furthermore, the rapid development of agricultural biotechnology and the release of new transgenic varieties have made ecological risk assessment of GM crops on the environment extremely important and also an urgent task (Ammann, 2005; Mocali, 2010).

Several studies have been carried out in order to evaluate the potential unintended effects of genetically modified plants on the environment and non-target organisms (Bruinsma *et al*., 2003; Saxena *et al*, 2004; Icoz and Stotzky, 2008). Although the issue is still controversial in many countries, especially in Europe where there is a level of continuing debate and public concerns (Drobnik, 2008), insect-resistant varieties including the 'stacked crops' with multiple traits occupied around 36% of the biotech area in 2009 (James, 2015). GM crops is only allowed in the field after undergoing an indepth environmental risk assessment among which is whether the GM crops have a different effect from the non GM crop on the soil microbiome, insects and neighboring plants of which have been the subject of scientific study for over 30 years (Nicolia *et al*., 2014). However, there are still concerns relating to this potential unknown effects of GM crops on the environment and a well defined risk assessment is still required (Wolfenbarger and Phifer, 2000; Bruinsma *et al*, 2003; Liu, 2009).

In Nigeria, some GM crops have been approved after series of field trials in October, 2018 which are *Bt* cotton and *Bt* maize though not yet released for commercial purpose as at the time of this research. Owing to this, there is need to generate indigenous data on the effect of GM crops on physiochemical properties of soil in Nigeria.

## 2.0 LITERATURE REVIEW

### 2.1 History of Genetically Modified Crops

The area of biotechnology that involves the manipulation of the genetic materials of living organisms thereby making such organisms perform specific functions is known as Genetic modification (Zhang *et al.,* 2016; Raman, 2017; Nalluri and Karri, 2020). The initial knowledge of modification of plants for domestic and consumption purposes dates back to approximately 10,000 years where scientists practiced "artificial selection" and "selective breeding", that is, selection and breeding of parent organisms having desirable characteristics (Doebley *et al.,* 2016; Zhang *et al.,* 2016; Raman, 2017; Nalluri and Karri, 2020). The use of these methods lead to a dramatic alteration

of plant genetic make-up which occurred through artificial selection of corn – from a weedy grass which possess tiny ears and few kernels (teosinte; Southern Mexico 6300 years ago) to the current cultivars of edible corn and maize plants (Singh *et al.,* 2015; Doebley *et al.,* 2016; Raman, 2017). Current variants of apples, broccoli and bananas different from their parent plant forms which are desirable for human consumption has also been reported through the use of similar techniques (Rangel, 2015; Raman, 2017).

The Flavr Savr tomato became the first ever approved GM plant for human consumption in USA by the Food and Drug Administration (FDA) in the year 1994 which was genetically modified by antisense technology to interfere with polygalacturonase enzyme production thereby causing delay in ripening and rot resistance (James, 2011; Rangel, 2015; Kamle *et al.,* 2017; Raman, 2017; Jan and Shrivastava, 2017; Nalluri and Karri, 2020). Several other transgenic crops were also approved for large scale human production ever since including, corn/maize, cotton, potatoes, canola, soybeans and most recently alfalfa, the GM crop technology has also expanded to cover fruits, cereals and vegetables such as lettuce, strawberry, eggplant, sugarcane, rice, wheat, carrots, pawpaw and so on with planned uses to increase bioproduction of vaccines, animal feed nutrients as well as improving salinity and drought resistant traits for plant growth in unfavourable environment and climates (James, 2010; James, 2011; Gruissem, 2015; Rangel, 2015; Zhang *et al.,* 2016; Raman, 2017; Jan and Shrivastava, 2017; Nalluri and Karri, 2020). GM crops have been beneficial to both the economy and environment since they have been commercialized, the food crop yield globally has increased by over 370 million tonnes over a relatively small acreage area (James, 2011; Singh *et al.,* 2015; Zhang*et al.,* 2016; Raman, 2017). Also, GM crops have reduced environmental and ecological impacts and has led to species diversity increase (Gruissem, 2015; Rangel, 2015; Zhang *et al.,* 2016; Raman, 2017). In October 2018, Nigeria joined the many other countries that have approved GM crops by approving GM cotton and maize after series of field trials, though it has not been commercialized as at the time of this research report.

### 2.2 General Procedure for Genetic Modification of Plants

There are few general steps followed in the genetic modification of plants, these include;

i. **Selection**
In genetic engineering, it is very important to first of all select the plant or other organism that contains the gene of interest that will be used to modify the desired plant.

ii. **Location**
Secondly, the gene of interest will be located and identified from the specific plant then cut out from the DNA using restriction endonuclease enzymes.

iii. **Multiplication**
This involves the production of multiple copies of the gene of interest which is then attached to a carrier or vector. Commonly used is soil bacterium, *Agrobacterium tumefaciens.*

iv. **Selection of transformed cells**
Selectable marker gene with a plant transcriptional promoter is used to select transformed cells.

v. **Culturing**
The plant cells that are to be modified are then cultured along with the *Agrobacterium* carrying the recombinant T-DNA with both a selectable marker and a desired transgene.

vi. **Injection**
In the culture media, some chemical substances particularly acetosyringone are released by the wounded plant cells which attracts the *Agrobacterium* then causing them to inject the gene of interest into the cells.

vii. **Proliferation**
Surviving cells that have taken up the appropriate DNA and express the selectable marker gene proliferate and form callus.

viii. **Acclimatisation**
The plant tissues that have taken up the gene of interest are then grown into matured plants and are called Transgenic or genetically modified plants (Gatew and Mengistu, 2019; Nalluri and Karri, 2020).

### 2.3 Physicochemical Properties of Soils Cultivated with Genetically Modified Crops

Soils cultivated with genetically modified crops are usually alkaline in nature, that is, pH of 8.0 to 8.6 with high electrical conductivity and low organic matter content compared to soils cultivated with conventional crops (Jeyabalan *et al.,* 2017). Just as soils cultivated with conventional crops and open soils, soils cultivated with genetically modified crops also consist of macro elements such as Nitrogen which usually changes with time and it is also the key environmental factor that affects the bacterial community of the soil used to cultivate the GM crop especially cotton, others include; Calcium, Magnesium, Sodium, sulfur, Phosphorus and Potassium and micro elements such as Zinc, Iron, Manganese and Copper all

within normal category (Tarafdar *et al.,* 2012; Lehman *et al.,* 2015; Jeyabalan *et al.,* 2017; Blaise and Velmourougana, 2017; Winsome, 2017; Tian *et al.,* 2020).

### 2.4 *Bt* Crops and Soil

There are several considerations on how *Bt* crops could affect soil microbes; they include both direct and indirect effects (Icoz and Stotzky, 2008; Mocali, 2010). The direct effects depend on the impacts of the DNA or proteins released from the modified crops on soil microflora. In contrast, indirect effects are mediated by changes in plant tissues and root exudates composition that could determine alterations of soil organic matter and microbial diversity with unpredictable consequences on soil quality and sustainability. Microbial communities represent more than 80% of the total soil biomass, excluding plant roots (Mocali, 2010), and perform many essential functions in the soil system such as organic matter decomposition and humification, redox reactions, Nitrogen fixation and solubilization, nutrient mineralization and immobilization (Nannipieri *et al.,* 2003; Mocali, 2010; Tian *et al.,* 2020). Therefore, any change in microbial functional or genetic diversity could lead to unknown consequences for the soil ecosystem (Lynch *et al.,* 2004; Mocali, 2010; Tian *et al.,* 2020).

## 3.0 MATERIALS AND METHODS

### 3.1 Study Area

The study was carried out at Genetically Modified Crop Screen House of Plant Improvement Unit, Agricultural Biotechnology Department, National Biotechnology Development Agency (NABDA), Abuja, the capital and eight most populous city of Nigeria with estimated population of 1,235,880 in 2011 and Federal Capital City and Municipality area of 1,769km$^2$ on $9^0 4^1 0^{11}$N $7^0 29^1 0^{11}$E coordinates (Lat. $9.08^0$ and Long. $7.49^0$) (Fig. 3.1), the indigenous inhabitants are the Gbagyis and their major occupation is farming. Abuja is bordered by the states of Niger to the west and northwest, Kaduna to the northeast, Nassarawa to the east and south and Kogi to the southwest.

### 3.2 Collection and Processing of Samples

The genetically modified (GM) cotton seeds MRC 7361 BG11 were obtained from National Bioseafety Management Agency (NBMA), Abuja, Nigeria as it has not been commercialized as at the time of this research while the non GM cotton seeds were obtained from the seed bank of Tissue Culture Unit of NABDA.

The seeds were planted (GM and Non-GM cotton) in twenty (20) four litter (4L) octagonal pots (one seed per pot) without added manure in the screen house due to the sensitivity of the GM cotton seeds. The pots were filled with four kilograms (4kg) of soil dug from the Tissue Culture Laboratory Unit of National Biotechnology Development Agency (NABDA), Abuja soil deposit and placed on bench in two rows of ten pots each and a third row of five pots (total of twenty-five pots), first row pots were used to plant GM cotton seeds, while the second row pots were used for non GM cotton seeds and a third row of five pots served as control, all in the screen house.

The physical and chemical properties of the soil samples (from the Plant Improvement Unit Soil Deposit) was done before filling the pots and after harvest for both GM and Non-GM cotton as well as the initial soil (in the screen house) which served as control at the Chemistry Laboratory Unit of Sheda Science and Technology Complex (SHESTCO), Abuja, Nigeria (Amorim *et al.,* 2008, Tarafdar *et al.,* 2011).

### 3.3 Determination of Moisture

Oven drying method was used to determine the moisture content of the soil as follows;

Ten grams (10g) of the soil sample was weighed, and oven dried at $105^O$C for 24hrs, the dry weight was then taken until it became constant. Weight loss corresponded to the water content of the soil.

% Moisture content = loss in weight on drying (g) / initial soil weight (g) x 100 (Wodaje and Alemayehu, 2015).

### 3.4 Determination of pH

Twenty grams (20g) of air dried soil was mixed with 50ml distilled water, then stirred with glass rod for 15 minutes and allowed to stand for 40 minutes. The electrode of the pH meter was then standardized using buffer solution of pH 7.0 after which it was inserted into the solution and the readings were taken (Wodaje and Alemayehu, 2015).

### 3.5 Determination of Nitrogen

Twenty grams (20g) of soil sample was weighed into a digestion flask and 100ml of 0.32% $KMnO_4$ solution, 100ml of 2.5% NaOH solution and 20ml of water was added, the flask was then connected to a standard distillation unit and 75ml of the distillate was collected in 25ml of boric acid indicator mixture (bromocresol green and methyl red), the absorbed Ammonia was then titrated with 0.05 $NH_2SO_4$ to calculate the Nitrogen content (Chaudhari, 2013).

### 3.6 Determination of Phosphorus

Five grams (5g) of fine soil sample was weighed into a clean and dry plastic bottle and 50ml of 2.5% acetic acid solution was added, then mixed manually for 2 minutes. The solution was kept for 3 hours until it was separated, 5ml of the supernatant was then measured into a glass bottle containing 5ml of colour developing reagent and 5ml of distilled water. The solution was mixed properly and kept for 15 minutes after which a blue colour developed, the blue colour intensity was then measured using the colour chart. This shows the phosphate level in the soil (Chaudhari, 2013).

### 3.7 Determination of Potassium

The potassium in the sieved dry soil samples was extracted using 0.5mol/L $NaHCO_3$ (pH 8.5) at a soil to solution ratio of 1:100 for 16 hours, exchangeable potassium was then eluted using 1mol/L $NH_4OAc$ at soil to solution ratio of 1:10 for 30 minutes. The eluted potassium was then analyzed by spectrophotometer at wavelength of 766,460nm (Chaudhari, 2013).

### 3.8 Statistical Analysis

The physicochemical parameters results were tested with ANOVA at P-value 0.05 to show either or not significant differences existed in the values.

## 4.0 RESULTS AND DISCUSSION

### 4.1 Results

The physicochemical parameters selected for analysis where; pH, moisture, Nitrogen, Potassium, Phosphate, water holding capacity and soil texture. The pH for the initial, GM cotton, non GM cotton and control soil samples were 6.28, 6.26, 7.25 and 8.26 (Tab. 4.1) respectively which shows that the GM cotton and initial soil samples were slightly acidic while non GM cotton and control soil samples were alkaline. The moisture for GM cotton, non GM cotton and control soil samples was observed to increase slightly from the initial soil sample which was 0.63%. Nitrogen was observed to be 17.70% for initial soil sample which decreased drastically for the other samples but Potassium on the other hand was observed to increase greatly for GM cotton (1,284.47mg/kg), non GM cotton (1,785.48mg/kg) and control soil (1,252.83mg/kg) samples compared to that of initial soil sample which was 0.46mg/kg. Water holding capacity for initial, GM cotton, non GM cotton and control soil samples were observed to be 1.96%, 1.89%, 1.80% and 1.93% respectively which shows that the soil samples were loose and the percentage of silt and clay observed showed that the soil samples was silty clay.

**Table 4.1. Physicochemical properties of initial and control soil and soil cultivated with GM and non GM cotton**

| Parameters | Initial Soil | GM cotton soil | Non GM cotton soil | Control soil |
|---|---|---|---|---|
| **Ph** | **6.28** | **6.26** | **7.25** | **8.26** |
| **Moisture (%)** | **0.63** | **0.78** | **0.89** | **0.82** |
| **Nitrogen (%)** | **17.70** | **1.14** | **1.10** | **0.56** |
| **Potassium (mg/kg)** | **0.46** | **1,284.47** | **1,785.48** | **1,252.83** |
| **Phosphate (mg/kg)** | **18.76** | **17.76** | **16.87** | **15.23** |
| **Water holding capacity (%)** | **1.96** | **1.89** | **1.80** | **1.93** |
| **Soil texture** | | | | |
| **Silt (%)** | **34.21** | **34.21** | **32.09** | **34.66** |
| **Clay (%)** | **58.89** | **58.89** | **60.23** | **59.23** |
| **Sand (%)** | **6.90** | **6.90** | **7.68** | **6.11** |

**4.1.1 Statistical Analysis for physicochemical parameters**

The sum of squares was calculated for groups as well as the mean of squares for each group to check if there is any significant difference between groups, the physicochemical parameters of soil samples at P-value less than 0.05 (Tab. 4.1) shows there was no significant difference between groups of samples.

**Table 4.2. ANOVA table for physicochemical analysis**

| | | Sum of Squares | Df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Initial Soil | Between Groups | 3033.446 | 8 | 379.181 | . | . |
| | Within Groups | .000 | 0 | . | | |
| | Total | 3033.446 | 8 | | | |
| GM Cotton soil | Between Groups | 1433287.978 | 8 | 179160.997 | . | . |
| | Within Groups | .000 | 0 | . | | |
| | Total | 1433287.978 | 8 | | | |
| Non-GM Cotton soil | Between Groups | 2786213.047 | 8 | 348276.631 | . | . |
| | Within Groups | .000 | 0 | . | | |
| | Total | 2786213.047 | 8 | | | |
| Control Soil | Between Groups | 1363148.192 | 8 | 170393.524 | . | . |
| | Within Groups | .000 | 0 | . | | |
| | Total | 1363148.192 | 8 | | | |

**KEY:**
**Initial Soil – Samples G and H**

**GM Cotton Soil – Samples 1A, 2A, 2B, 3A and 3B**

**Non-GM Cotton Soil – Samples 1C, 1D, 2C, 2D and 3C**

**Control Soil – Samples 1E, 1F, 2E, 2F and 3E**

**4.2 Discussion**

Crops modified with herbicides tolerance, disease resistance, insect/pest resistance, drought tolerance and salt tolerance genes gives superior agronomic traits and improved product quality (Nalluri and Karri, 2020; Tian *et al.,* 2020). Assessing the effects of GM crops on microbial ecology and physicochemical properties of soil is important as there may be unexpected potential effects on the environment and human health. After all, with GM technology, traits that were not initially present in crops may now be obtained which may have direct or indirect effects on the environment due to different methods of cultivation (Mocali, 2010; Sanvido *et al.,* 2012; Tian *et al.,* 2020).

pH for the soil samples were slightly acidic (GM cotton and initial soil samples) and alkaline (non GM cotton and control soil samples) while the percentage Nitrogen was moderate for GM and non GM cotton and control soil samples and high for initial soil sample. This may be responsible for the abundance of the Terrabacteria, Proteobacteria and Archaea groups as they can survive extreme conditions and are responsible for fixing Nitrogen in the soil. The low moisture content may be due to the fact that the set-up was in the screen house where there was no direct rainfall, other parameters such as Potassium and Phosphorus varying concentration and low water holding capacity may be traced to the soil texture which was silty – clay. The physicochemical properties results when tested with ANOVA at less than 0.05 P-value showed no significant differences. According to the results, it can be deduced that the genetically modified crops had no apparent effect on the physicochemical properties of soil and thus may not have any adverse effect on the environment and in turn no direct or indirect effect on human health. Though, this research work has shown that there is no apparent effect of genetically modified crops on the physicochemical properties of soil, there is still need to assess the potential effect of GM crops on the soil environment.

## 5.0 CONCLUSION AND RECOMMENDATION

### 5.1 Conclusion

Nigeria has joined the list of countries growing GM crops by approving Bt cotton and Bt maize after several field trials in October, 2018. The pH for initial and GM cotton were acidic while non GM cotton and control soil were alkaline, the percentage moisture for GM cotton, non GM cotton and control soil was slightly different from that of initial soil while the percentage Nitrogen was observed to be high for initial soil and moderate for the other soil samples. Other parameters include, varying concentrations of Potassium which was high for GM cotton, non GM cotton and control soil samples but low for initial soil sample and Phosphorus which was not too different for the four treatments. The soil texture was silty – clay as the silt and clay percentages were higher than that of sand.

Though many other studies suggested that BT plants cause minor changes in the physicochemical properties of the soil, this research has proved otherwise since there is no apparent effects on the physicochemical properties of the soil. This suggests that there are no significant effect on the environment as well as direct or indirect effects on human health.

### 5.2 Recommendation

It is recommended that:

*Bt* Cotton should be accepted and commercialised as it has no apparent effects on the environment as well as on human health.

Irrespective of the positive outcome of this research project, further research on GM crops and their potential effects on the environment should be encouraged and carried out.

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