INTERNATIONAL JOURNAL
Research in
Biological Science
www.gphjournal.org || editor@gphjournal.org

Original Research Paper | Vol. 04 Issue 11 Nov - 2021 | Manuscript ID: #0517

# EVALUATION OF PRIMING ON ANTINUTRITIONAL FACTORS IN LEAF OF CUCURBITA MAXIMA IN RIVERS STATE NIGERIA

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## ABSTRACT

The capacity of priming agents on the management of antinutritional factors in the leaf of Cucurbita maxima (duchesne Linn) winter squash was evaluated using hydro-prime agent (Biomagnetic treated water) and Bioprime agents (Biochar and Bacillus licheniformis with control at Rivers State University Teaching and research farm Port Harcourt, the leaf of Cucurbita maxima primed plant was harvested and further processed for the reduction of antinutritional factors using standard methods. The harvested data was subjected to ANOVA of variance-(DUNNETT and Turkey means comparison), Microsoft test and Statistical analysis system-john Macintosh project. However, the test results inferred that Biomagnetic treated water and Bacillus licheniformis had highest positive and negative percentage difference while biochar treated sample showed equilibrium in both negative and positive percentage different but reduced more negatively on phenol antinutritional value all on the leaf and seed sample plant. Moreover, the variational analysis confirmed the efficacy of biomagnetic treated water followed by Bacillus licheniformis and biochar sample plant informed importance of ipomea aquatica as biomass for biochar production verified via sequenced result obtained.

## KEYWORDS

*Evaluation of priming, antinutritional factors, Cucurbita maxima, leaf.*

## Introduction:

**What is Priming:** These are treatment process subjected to seeds of plants for a period of three days to remove the plant seeds from dormancy before sowing thereby allowing the seeds to undergo imbibition to bridge the prolonged process of early-stage germination disallowing protrusion of radical(Pawar & Laware, 2018; Pirasteh-Anosheh & Hashemi, 2020). However, Priming could be assessed as medium through which seed early germination is biocontrol to regulated cellular cycle, uniform molecular development, moisture control, epigenetic control, mitigate stress, molecular cellular repairs and enhance positive mutation thus influence the reduction of secondary metabolites in leaf of plant(Barbosa et al., 2016).

**Secondary Metabolites:** Antinutritional factors are synthetized from primary metabolites: -lipids, amino acids and carbohydrates in most higher plants(Jan et al., 2021). They serve as food additives, flavour, ethnomedical sources, biocontrols, and epigenetic mitigations. However, their production mainly depends on the plant growth, physiologic and developmental phases also their accumulation is mostly due to epigenetic factors(Jan et al., 2021). Moreover, high bioaccumulation of antinutritional factors can be reduced via germination, sprouting, boiling priming agents and roasting(Moghadam et al., 2016). Antinutrients are naturally or artificially occurring biochemical substances in plants and animal systems that could mask the bioavailable mineral nutrients absorption process thereby mitigate the nutrient intake, utilization and digestion(Popova & Mihaylova, 2019). Thus, some side effect due to high consumption of vegetable are mostly attributed to bioaccumulations of secondary metabolites resulting to rashes, bloating, nausea, malabsorption nutritional deficiencies while monogastric animals are weak in reducing high bioaccumulation of antinutrient when consumed unprocessed moreover, when ingested in a right ratio would serve a defensive substance for plant and monogastric animals(Popova & Mihaylova, 2019; Samtiya et al., 2020). The accurate dosage for antinutrient accumulation is still not clear based on the band by USA FDA and UKBA on the use of some antibiotics as a food meal for domesticated animals in-order to reduce drug resistance diseases**(Ebrahim, 2020; Ehsen et al., 2016). The effect of priming agents in the reduction of Antinutritional factors in Cucurbita maxima leaf:** Most priming agents are eco-friendly, cheap, and can be accessed in every corner of our surrounding one of such is Hydropriming where seeds are socked in water in absent of chemical addition(Damalas et al., 2019)

**Magnetopriming agents**: this could be classified as a process were molecular structure of liquid is broken down with no depose of any chemical substances or impurity but reduction of giant molecular of water by the use of bio-disc fix in a biorotor to covert the scaler energy into a potential energy which can aid the redox reaction of giant molecular structure of water to hydroxyl ion that serves was carrier of valences ions into chlorophyl and nucleus of the plant cells(Korotkov, 2019). Hence the restructuring of the giant molecule of water could allow easy penetration of charged ions in and out of cellular surfaces thereby breaking the molecular structure of antinutritional factors and contaminant in the aqueous solutions present in any environment hence reducing the amount of bioactive substances(Obaroh et al., 2016a).

**Biopriming agents as antinutrient stabilizers**: these are those agents that are extracted from biological sources or colony of microbial inoculum and serving as stable substrate for treatment of seeds before pre-sowing disallowing protrusion of radicles they are sometimes classified as microbial extract, nutritional extract, and plant biomass for biochar other than synthetic chemical substances(Chin et al., 2021). The rate of germination of seeds is dependent on the bioagent used, *Bacillus licheniformis* broth could compete with biochar source, although the biomass use for the biochar production could also contribute to the different growth parameters and bioactive substance found in the plant. However, bacillus licheniformis could help in imbibition of water and charge soluble molecules hence bioregulate antinutritional substance to different part of the plant(Akhtar et al., 2020) during alternate environmental condition. Biochar, coating of seeds could also bioregulate seeds metabolism when applied during pre-sowing of seeds hence supply molecular nutrient to break dormancy in seeds thus reducing germination lag time thereby improving the leaf area index and photosynthesis, respiratory and bioaccumulation of bioactive and mineral nutrient of the plant(Kiruthiga et al., 2019). There are high antinutritional factors in Cucurbita maxima and are eaten raw bleach also fleshly consumed by vegetarians while the bioaccumulation of the antinutrients can lead to kidney failure, diarrhea, nausa

## Materials and Methods

### EXPERIMENTAL LOCATION:

Rivers State University Biology Laboratory and Research farm where use for priming and field trials. GCMS were carried out in University of Port Harcourt Regional Centre for Bioresearch (RCBBR).

### SAMPLE COLLECTION AND PREPARATION:

#### Biopriming Agents:

Biochar was prepared in Rivers State University biology laboratory using *Ipomoea aquatica* (water spinach-swampy morning glory) in a mini oven at temperature of 350 $^{o}$C for 2 hours and *Cucurbita maxima* fruit was purchase from Port Harcourt Town market and were identified by Green O. Blessing. Seeds from matured fruits of *Cucurbita maxima* were extracted from the pulp, washed and air dried for 20 minutes and viable test was done to select seeds that were good for pre-treatment. *Bacillus licheniformis* pure culture was gotten from international institute tropical agriculture IITA Ibadan, viable cells were revived by subculturing the colonies in a nutrient agar plate, the broth were prepared and used as biopriming agent.

#### Hydropriming Agents

Biomagnetic treated water were prepared in Rivers University biology Laboratory by the conversion of the Bio-disc scaler energy to potential energy by the help of rotor machine.

However, the pre-treated seeds were covered with Whatman paper, and then were transferred to the research farm for field trial. Irrigation was done once a day for four weeks while flowering and fruiting started from 60 days of sowing.

### GAS CHROMATOGRAPHY AND MASS SPECTROSCOPY

Ten leaves were harvested in triplicate from each ridge of treatment and immediately put into a cool icebag labeled properly and were transported to University of Port Harcourt Regional Centre for Bioresearch's (RCBBR) for processing.

### MATERIALS AND METHOD FOR GC/MS

Agilent 6890N Gas Chromatograph with Agilent 5975 Mass Selective Detector(Llorente et al., 2019).

#### Equipment, Materials, and Reagents

Auto sampler vials, 150 µL vial inserts, and crimp seals
Vial crimper and De-crimper
2.5mL airtight syringe or 3mL disposable hypodermic syringe
10 micro-liter autosampler syringe
30-m x 0.25-mm or 0.32-mm ID fused-silica capillary column
chemically bonded with SE-54 (DB-5 or equivalent), 1-µm film thickness.
Soxhlet extractor.

#### REAGENTS

Air–Zero grade
Helium gas –UHP grade
n-hexane
Anhydrous sodium sulfate
Standard plant chemicals (internal standard)

**Sample Preparation:** A 20-g aliquot of sample is homogenized, and a 10-g aliquot is spiked with the labelled compounds. The sample was mixed with anhydrous sodium sulfate, allowed to dry for 30 minutes minimum, and extracted for 18 - 24 hours using methylene chloride in a Soxhlet extractor. The extract is evaporated to dryness, and the lipid content was determined.

**Procedure:** Extracts for phytochemical analysis were subjected to a sequential methylene chloride-n-hexane (1:1) clean up specifically for these analytes, 1µL of the sample was injected into a gas chromatograph

equipped with either a narrow-or wide-bore fused-silica capillary column and either an electron capture detector (GC/ECD) or an electrolytic conductivity detector (GC/ELCD).GC–MS analysis was carried out using an Agilent 6890 gas chromatograph with a 5975, Mass Spectrophotometer (MS) detector equipped 30-m x 0.25-mm or 0.32-mm ID fused-silica capillary column chemically bonded with SE-54 (DB-5 or equivalent), 1-µm film thickness. (Agilent). The following temperature ramp was used: injector at 250 °C, oven initially at 200 °C, held for 1 min and heated to 230 °C (1.5 °C min-1, then held for 10 min). The characterization and identification of phytochemicals, from the sample was completed in the SCAN mode with the m/z range varied from 35 to 450. The flow rate of the helium as carrier gas was 1 mL min-1; manual injection; the injection volume was 1 µL. Interpretation of mass spectrum of GC-MS was done using database of National Institute of Standard and Technology (NIST). The mass spectrum of unknown component was compared with the spectrum of the known component stored in the NIST library. Major components were identified by with authentic standards and by with recorded from computerized libraries film thickness.

## RESULT

## ANTINUTRIENT ANALYSIS

**First Leaf Sample Antinutrient**

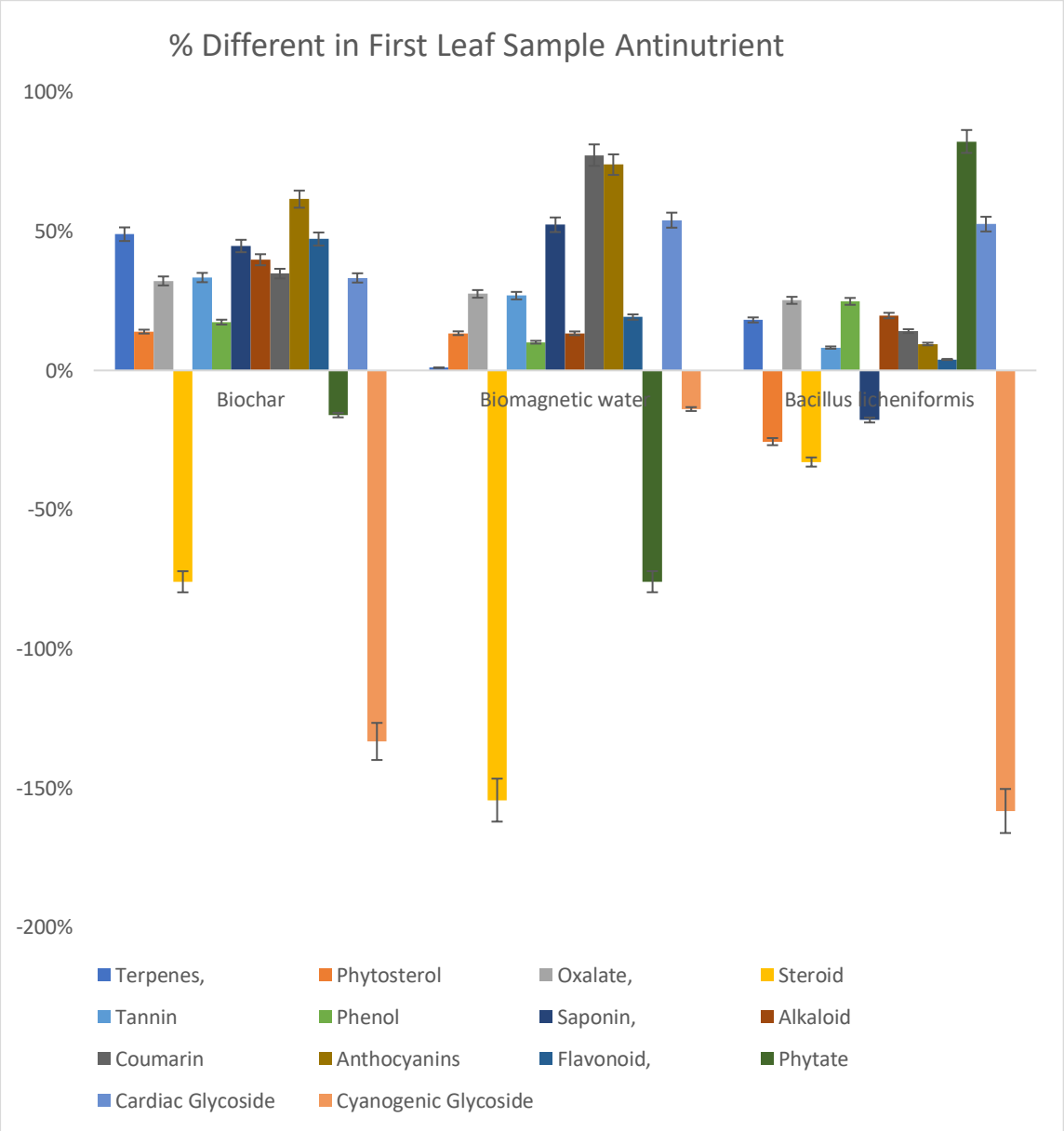

*Figure 1: % Difference in first leaf sample antinutrient*

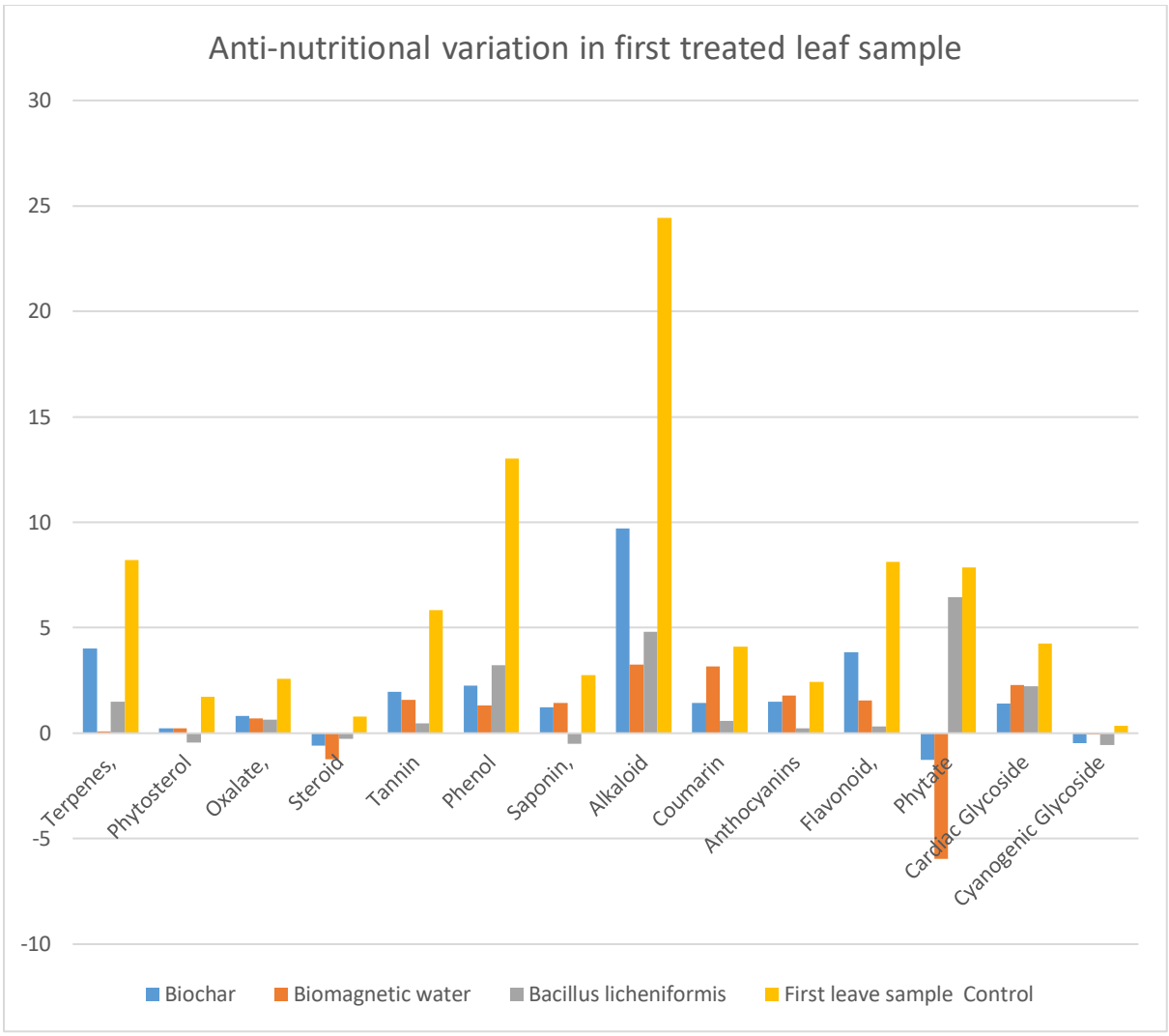


*Figure 2: First Leaf Sample Antinutrient Variation*

**Antinutritional sample from Second leaf of *Cucurbita maxima* analysis**

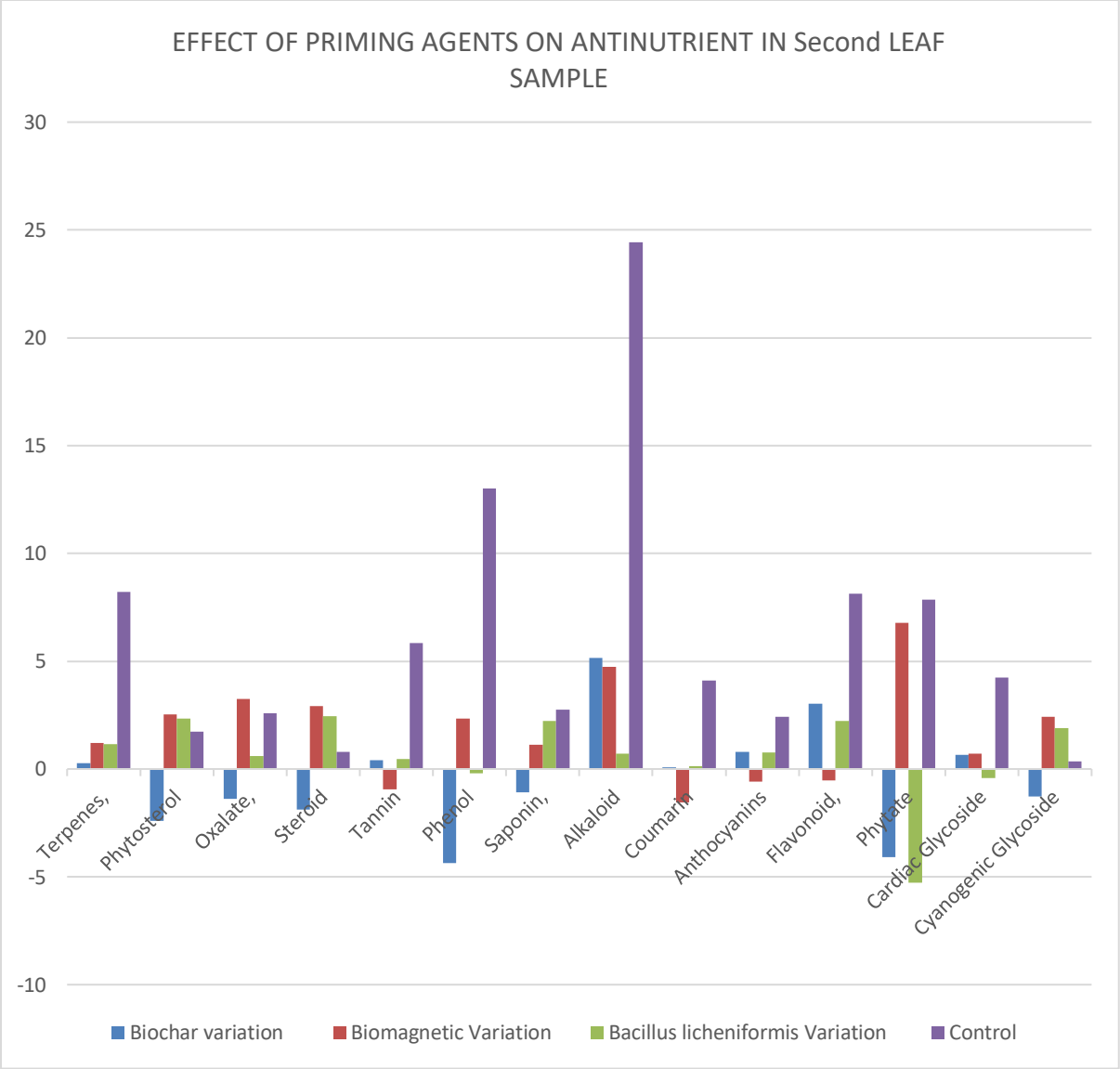


**Figure 3**: *Showing effects of priming agents on anti-nutritional variation status of second leaf of Cucurbita maxima sample*

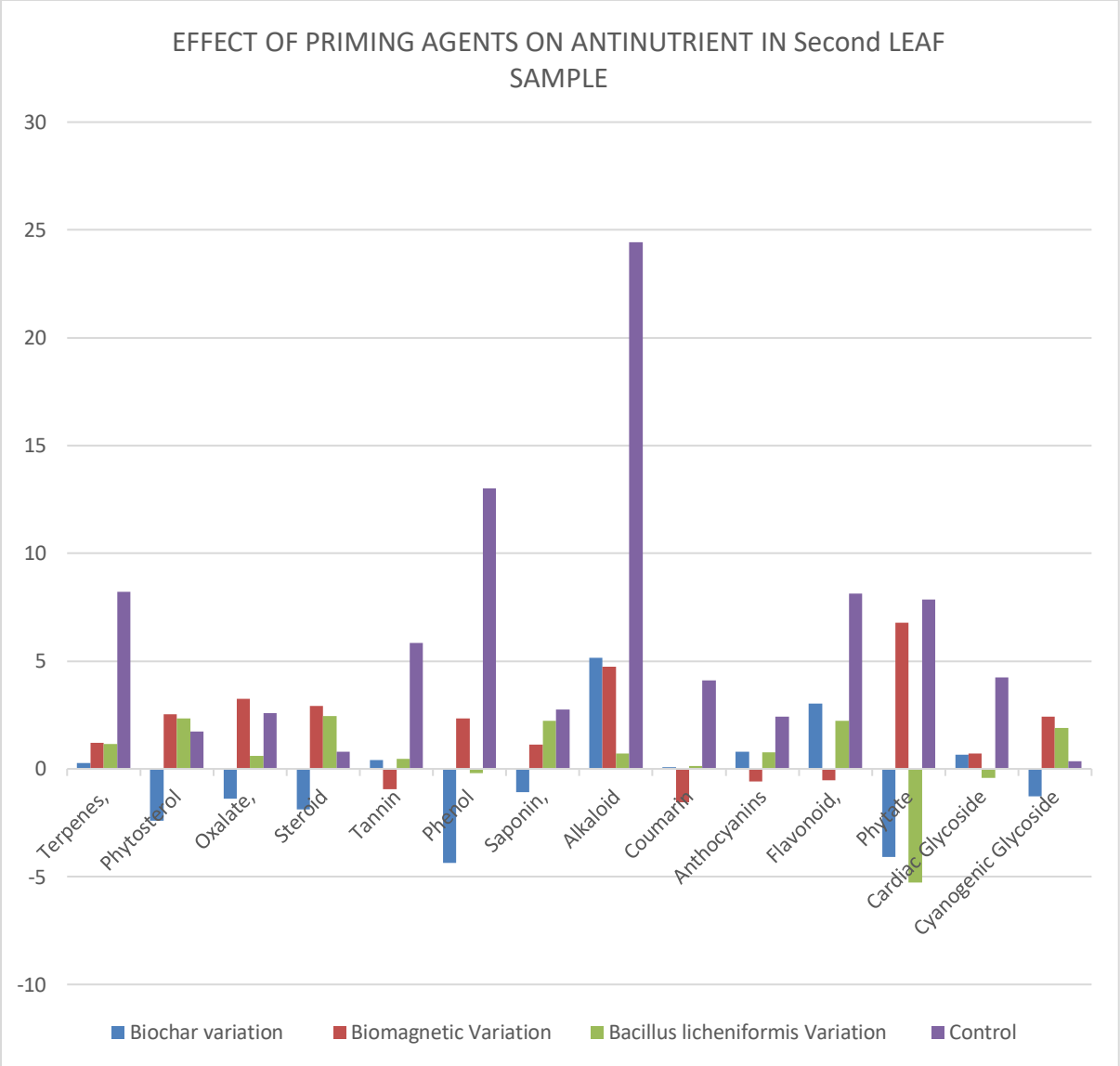


***Figure 4:*** *Showing % difference of antinutritional status of second leaf sample*

**Effect of priming agents on antinutritional status of third leaf**

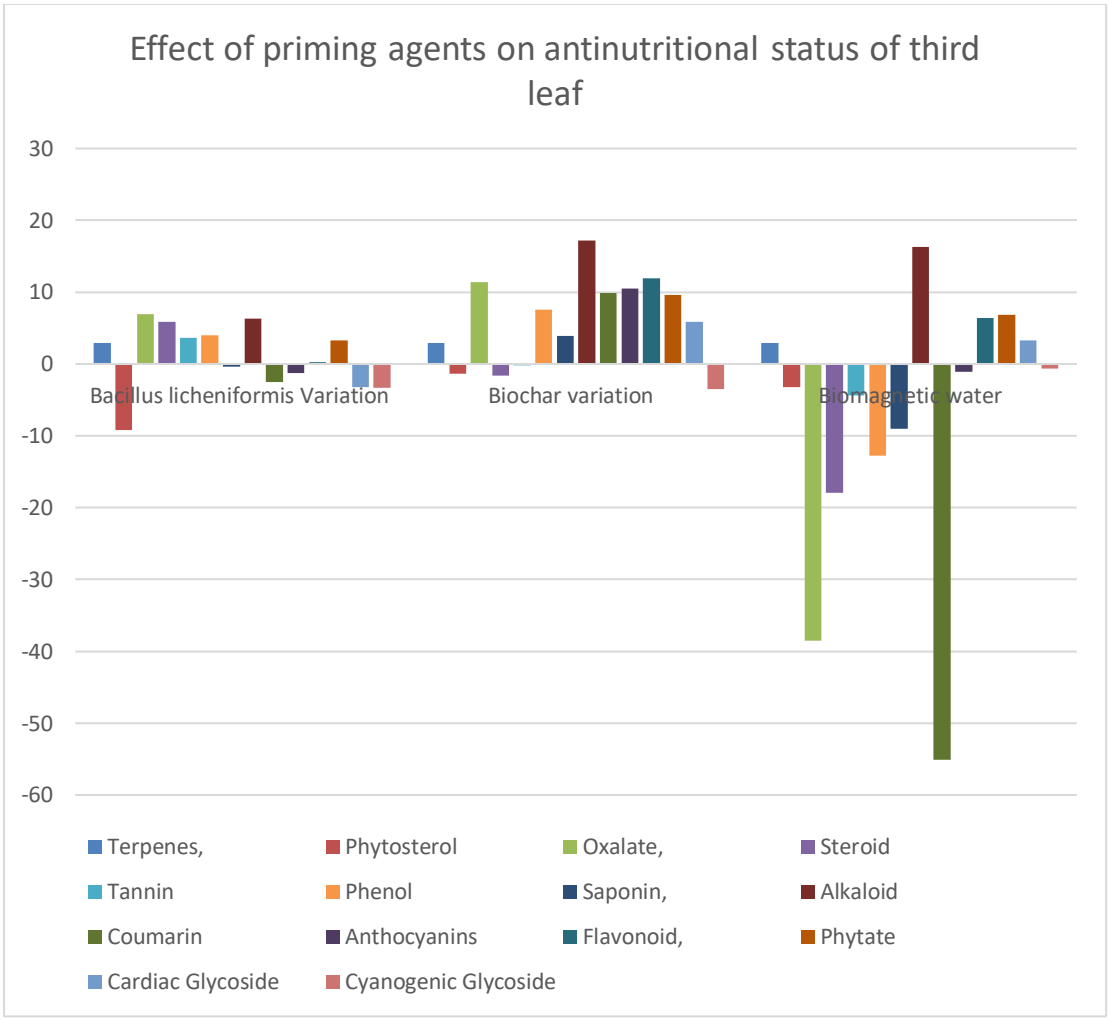


***Figure 5:*** *Showing Variation due to effect of priming agents on antinutritional status of third leaf*

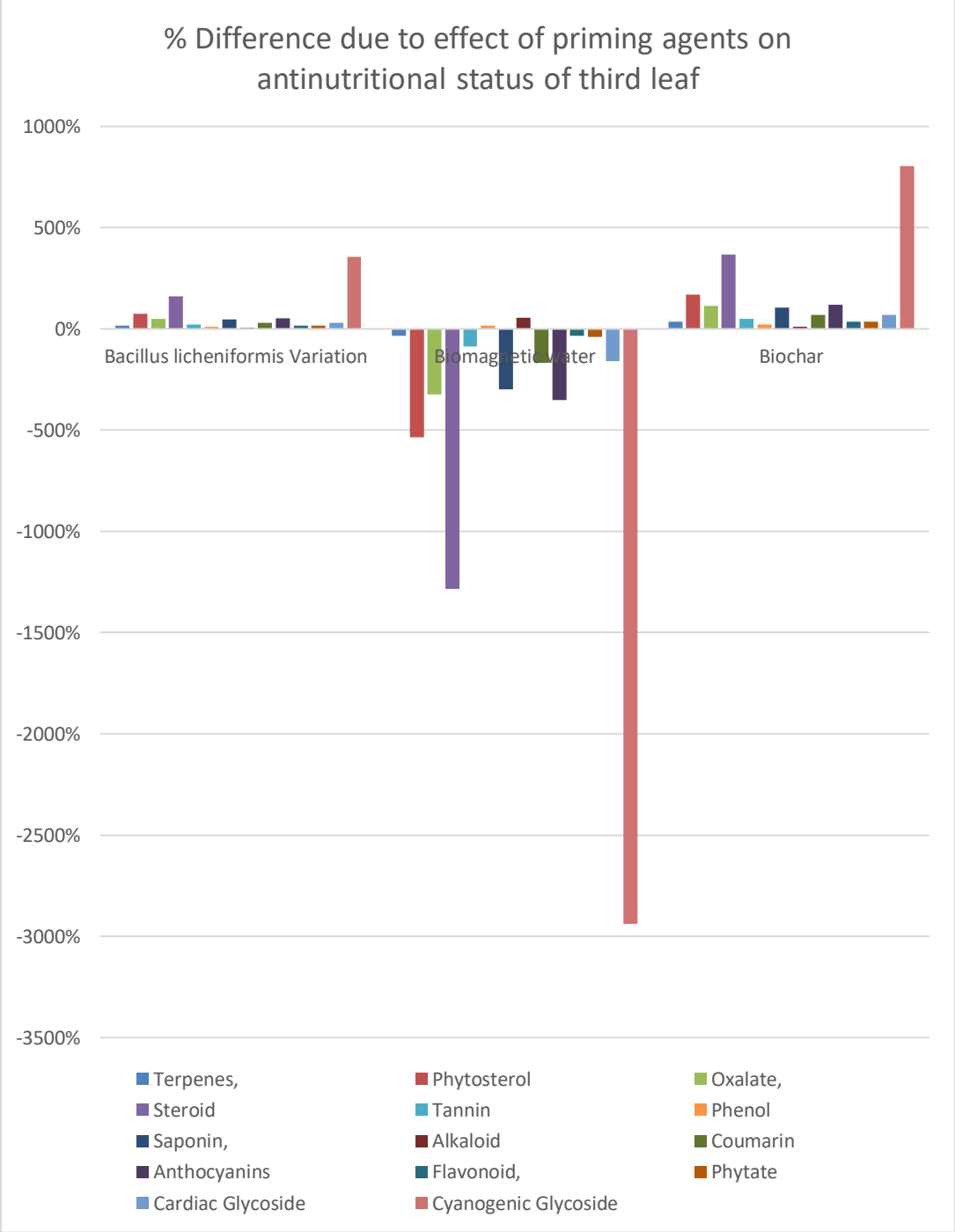


***Figure 6:*** *% Difference due to effect of priming agents on antinutritional status of third leaf*

## Discussion

### Effect of priming agents on antinutritional of first leaf samples:

Figure 2: Had *Bacillus licheniformis* priming agent taking highest at Alkaloid value followed in descending order <flavonoid <terpenes <cardiac glycoside <phytate <phenol <cyanogenic glycoside <saponin <tannin <steroid the antinutrients appearing in descending order(Falade, & Ojokoh, 2017).

Biochar primed agent: Alkaloid was highest followed by flavonoid appearing in descending order terpenes< oxalate< anthocyanins<cardiac glycoside< phytate <Phyto-steroid and Steroid as the lowest(Akachukwu et al., 2018). *Bacillus licheniformis*: The highest antinutrient was Alkaloid < Flavonoid < coumarin < anthocyanins < terpene < Oxalate < cardiac glycoside < Phenol < phytosterol as lowest. Figure 1: showed that biomagnetic water treated samples had highest negative reduction(Obaroh et al., 2016b) followed by biochar but biomagnetic water got medium in positive reduction while biochar sample had highest in positive reduction. Mainly *Bacillus licheniformis* got lowest in both negative and positive reduction in the first leaf sample inline the Bacillus licheniformis previous experimental treatment(Risan & Muhsin, 2015).

**Effect of priming agents on the antinutritional content of second leaf sample:**

Figure 3: showing *Bacillus licheniformis* primed sample had Cyanogenic glycoside as highest in descending order < steroid < flavonoid < saponins < Alkaloids <oxalate < Phyto-steroid < Terpenes < anthocyanins < phytate < cardiac anthocyanins < Tannins< phenol < cardiac glycoside as the lowest(Wojtyla et al., 2016) hence reduced the rate of antinutrient composition in treated plant but not as high as biomagnetic water sample plant.

Biomagnetic water priming agent samples: Alkaloid was highest next was Oxalate < Cardiac glycoside < flavonoid < terpenes < steroid < phytate < anthocyanins < saponins < phyto-steroid < tannins < phenol <coumarin as reduce highest in the level of antinutrient(Korotkov, 2019).

Biochar priming agent sample: Flavonoids< anthocyanins < alkaloid < cardiac glycoside < Tannins < Terpenes < Coumarin < saponins < Oxalate < phenol < cyanogenic glycoside < steroid < phyto-steroid increased highest in antinutrient level probably due to *ipomea aquatica* biomass sources(Tang et al., 2017) thereby improving microbial association.

Figure 4: Showed percentage difference of antinutritional status of second leaf sample, where biochar treated sample had highest negative reduction in line with the previous author supporting *Ipomea aquatica* action as biomass source(Guo et al., 2020), followed by biomagnetic treated water samples inline with(Korotkov, 2019) next was Bacillus licheniformis also biomagnetic treated water samples had highest positive reduction followed by *Bacillus licheniformis* treated samples least was biochar in positive reduction(Guo et al., 2020) in support with this previous writer.

**Effect of priming on third leaf antinutritional values**

Following the finding in figure 5: Biomagnetic water reduced more of the antinutrients in the negative direction followed by Bacillus licheniformis and biochar had less in the negative reduce in support with the capacity of biomagnetic treated water by bio-disc to change scalar energy to potential energy in any surface is placed via negative polar region rotation(Korotkov, 2019). Also Bacillus licheniformis having the potential to reduce antinutrients when inoculated or fermented in a substrates(Falade, & Ojokoh, 2017) while biochar reduced highest in positive direction in line with other researches(Akachukwu et al., 2018). Moreover, in figure 6: depicting % difference in reduction in the negative non positive direction biomagnetic treated water reduced the antinutrients present in the leaves more in negative direction(Obaroh et al., 2016b) than *Bacillus licheniformis* followed by biochar. However, biochar reduce more antinutrient in the positive direction probably due to the ability of *Ipomea aquatica* biochar source transferring its retained antinutrients via bioaccumulation thus, serving as a biofertilizer(Guo et al., 2020).

## CONCLUSION

Nonetheless, the antinutritional factors present in*Cucurbita maxima* can be managed by the used of hydropriming agent: Biomagnetic water, and Biopriming agents: Biochar ***and Bacillus licheniformis,*** However, amongst the priming agents used Biomagnetic water performed highest in reduction of the antinutrient, followed by ***Bacillus licheniformis*** but the Biochar increase theantinutrients. Moreover, the dosage of priming agents use can determine the rate of antinutrient reduced and bioregulation across the plant parts.

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