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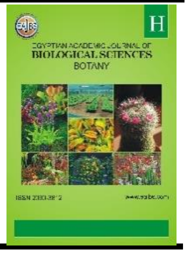


# Preparation and Characterization of Biochar and Their Effect on Cell Division

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**ABSTRACT**

The current investigation was carried out in the Agricultural Botany Department; Soil Department, Faculty of Agriculture Saba Basha, Alexandria university, Alexandria, Egypt in collaboration with Plant Protection and Biomolecular diagnosis Department, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications, Borg El-Arab, Alexandria, Egypt. The main objectives of the current study are preparing and characterization of different sources of biochar from maize, rice, and farm residues; an application of biochar with wheat seeds in soils to determine the effect on germination and other testes and study the effect of biochar on mitotic division and chromosomal aberrations. For biochar generated from straw rice, the results showed a high percentage of carbon compared with the other elements. The sample recorded many elements such as Carbon, Oxygen, Magnesium, Silicon, Potassium and Calcium. While the biochar from maize stalk includes higher elements compared with the rice straw biochar, the highest two elements were Carbon and Silicon, respectively. Finally, for biochar from tree residues, the analysis of the elements showed an increase in Carbon, Calcium and Oxygen, respectively compared with the other biochar types the Calcium was higher. During the current study, different biochar levels and sources effect were tested on the cell division and mitotic index besides their effect on chromosomal aberration on wheat root tips. The wheat root tips were collected and examined under microscopic (100x) to detect the several mitosis phases such as prophase, metaphase, anaphase, and telophase, in addition to cytokinesis. The chromosomal aberrations were observed and detected to show the effect of different biochar levels and sources such as stickiness, lagging chromosome bridge, multinuclei, ergon, abnormal prophase, abnormal metaphase, abnormal anaphase, elongation of nucleus content and gab chromosome.

## INTRODUCTION

The charred solid generated by thermal decomposition of organic materials in a limited oxygen environment, this process known as pyrolysis, is called biochar (Joseph *et al.*, 2010). Also, we need to identify that biochar is not a pure carbon (C) but it's including


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some different compounds such as ash (A), Hydrogen (H), Oxygen (O), Nitrogen (N), and Sulfur (S) as maintained by Duku *et al.* (2011) and Lehmann and Joseph (2015). In the last period, the usage of biochar in arable lands has got great awareness due to its agronomic and environmental benefits (Liu *et al.,* 2013 and Stavi and Lal 2013). Biochar's as soil improvement have been measured to mitigate universal warming, restore degraded lands, and balance water pollution by eliminating organic pollutants for instance pesticides, dyes, and pharmaceutical and personal care products (Barrow 2012; Inyang and Dickenson 2015). Furthermore, it can potentially inactivate *Escherichia coli* (*E. coli*) by decontamination, transform 95 % of 2-chlorobiphenyl by enhanced oxidation processes, (Inyang and Dickenson 2015), and increase the soil fertility and crop yield associated with biochar adding to different soils (Cayuela *et al.,* 2013 and Stavi and Lal 2013).

Biochar improves the soil's capacity to adsorb plant nutrients (Cheng *et al.,* 2008). It's can decrease the soil bulk density, and enhance cation exchange capacity, nutrient travelling, and the capacity of soils to preserve plant available water. Therefore, using biochar as a soil amendment is projected to improve both nutrient and water use effectiveness and crop productivity (Liang *et al*., 2006). In fact, there are several studies indicating that soil biochar applications enhance the crop yields such as (Yamato *et al.,* 2006). Biochar is thermally decomposed biomass purposely utilized in the soil to enhance its properties (Lehmann and Joseph 2015). By classification, biochar is extremely associated with charcoal, but the latter is fuel and not a soil amendment. But production processes for biochar and charcoal might be comparable, the first encompasses technically- enhanced processes created to produce kinds of clean, renewable energy production (Bridgwater, 2006)

Biochar product helps to improve crop productivity due to improving soil nutrient supply and activity of different microbe and reducing nutrient leaching (Liu *et al*., 2013; Ventura *et al*., 2013). Based on their liming effect, biochar improves the supply of essential macronutrients and micronutrients for plant development (Van Zwieten *et al*., 2010). Biochar as soil amendment improved the soil structure by increasing the soil porosity significantly (Lehmann *et al.,* 2003), enhancing water content, and enhancing nutrient holding in soil micropores (Lehmann and Joseph 2015). Biochar is generated from different sources such as crop residues, forest residues, sewage sludge, algae, and manure (Lehmann and Joseph 2015). Also, the pyrolysis processes showed a significant impact on biochar characters representing biochar yield, biochar pH, biochar particle size, and biochar surface area. Biochar commonly enhances the soil water content, cations exchange capacity, and C content (Lehmann *et al.,* 2012). Biochar generated from several feedstock resources or pyrolysis processes varies in pores size, pH, surface area and charge. (Ahmad *et al*., 2012a) and, thus, behave otherwise in contrasting soils owed to their varying adsorption behavior and biological activity (Fungo *et al.,* 2014).

Ahmad *et al*. (2014b) and Lehmann and Joseph (2015) reported that many feedstocks are used for biochar manufacturers such as the agricultural residues mapping and wood handling residues, algae, public solid waste, livestock/poultry waste, wastewater/sewage sludge, and biosolids. Biochar as a soil amendment may be the carrier of several dangerous compounds like HMs such as Cd, Cu, Cr, Ni and Zn as reported by Hospido *et al.* (2005), and additional toxins for instance volatile organic compounds, xylenols, cresols, acrolein, and formaldehyde (Kim *et al.,* 2015). The toxic materials are generated by the catalytic meeting of dioxin structures from Oxygen (O), Carbon (C), and Chloride (Cl) from 300 to 325 °C, frequently catalyzed by Iron (Fe) and Cu. Rogovska *et al*. (2012) showed that the germination and plant growth were reduced with biochar treatment due to the presence of several phytotoxic compounds, also, Rogovska *et al*. (2012) reported that biochar helped in improving plant germination. Though, not enough information is available to describe the role of biochar in plant germination, which also differs on soil type and pyrolysis process (Solaiman *et al.,* 2010). Solaiman *et al*. (2010) observed that various biochar and their treatment affected wheat seed germination and seedling growth rate, in addition, and the responses of mung bean and subterranean clover differed from that of wheat.

The main objectives of the current study are preparing and characterization of different sources of biochar from maize, rice, and farm residues; an application of biochar with wheat seeds in soils to determine the effect on germination and other testes and study the effect of biochar on mitotic division and chromosomal aberrations

## MATERIALS AND METHODS

**Experimental Place:**

The current investigation was carried out in the Agricultural Botany Department; Soil Department, Faculty of Agriculture Saba Basha, Alexandria university, Alexandria, Egypt in collaboration with Plant Protection and Biomolecular diagnosis Department, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications, Borg El-Arab, Alexandria, Egypt

**Biochar: Preparation and Characterization:**

Biochar is the remains byproduct of carbonaceous material converted by the thermal conversion of agricultural waste in the absence of oxygen by conducting anaerobic burning of organic matter at a high temperature of 700°C, which leads to rapid disposal odors and transformed into brittle coal**.** Biochar was prepared from three agricultural wastes: corn stalks, rice straw, and tree residues from the farm of the Faculty of Agriculture Saba Basha, Alexandria University for the 2021 season (Fig. 1).

The corn stalks, rice straw, and tree residues were washed carefully with tap water and then with distilled water to remove all impurities. After that, they were dried in a drying oven at 105°C for 24 hours until completely dried. The agricultural waste was placed in a sealed container in an anaerobic burning furnace to prevent entering the air inside the box at a temperature of 600°C for a period of three hours after this the furnace was left for 24 hours until reaching room temperature. If the burning box is not closed properly the air will enter and the material turns into ash. After this, the biochar was ground by a primary grinder and fine grinder to decrease the biochar particles to less than a half-millimeter. The biochar weight, density, and mass loss (%) were calculated at the end of the carbonization process.


**Fig. 1:** Different steps of biochar preparation from different sources.

**Seedling Sowing and Recorded Data:**

One seed one plant of wheat *Triticum aestivum* L. (Cultivars: Misr 1 and Sakha 95) were used in this study. Pots were prepared and mixed with different sources and levels of biochar from three agricultural wastes: corn stalks, rice straw, and tree residues from the farm as recorded in Figures (2-5) for the whole practices of sowing.

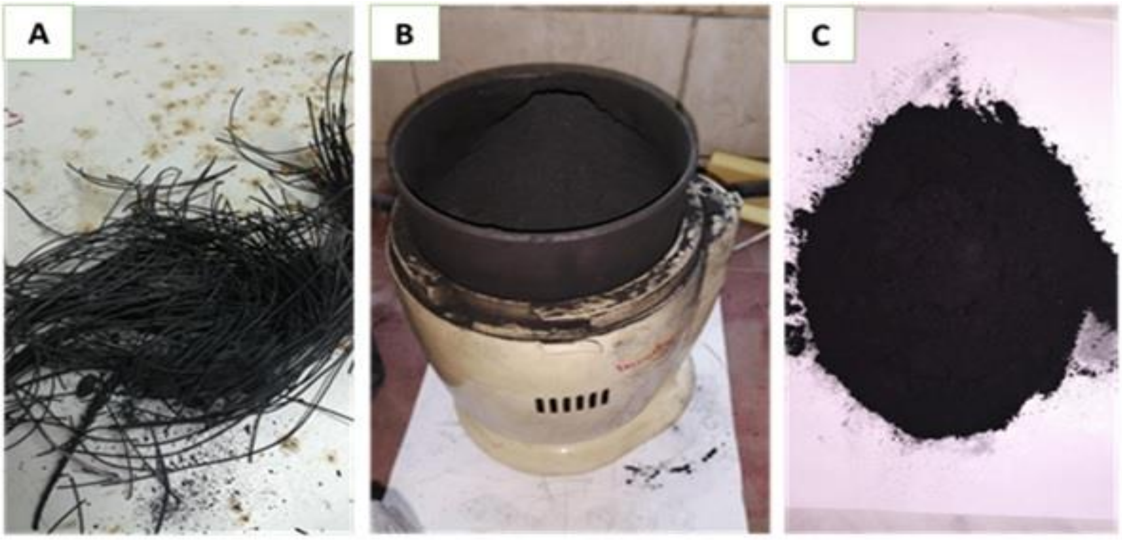


**Fig. 2:** An example of biochar preparation from different plant sources.

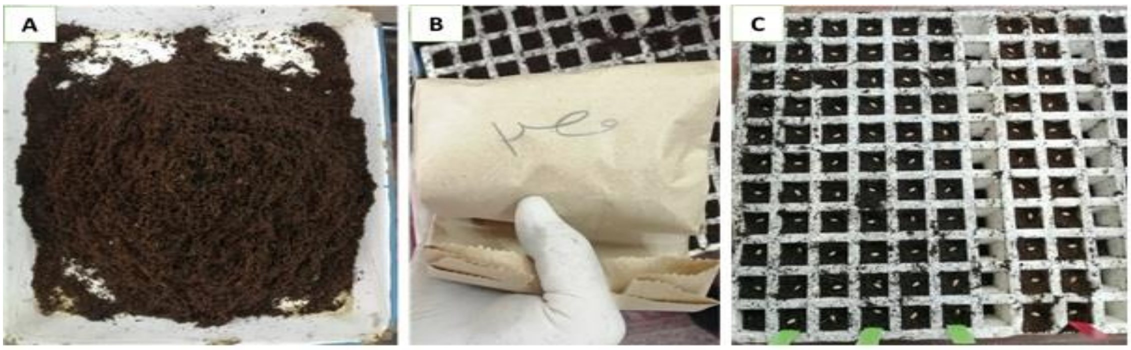


**Fig. 3:** Showing preparation of wheat grains (one seed one plant).

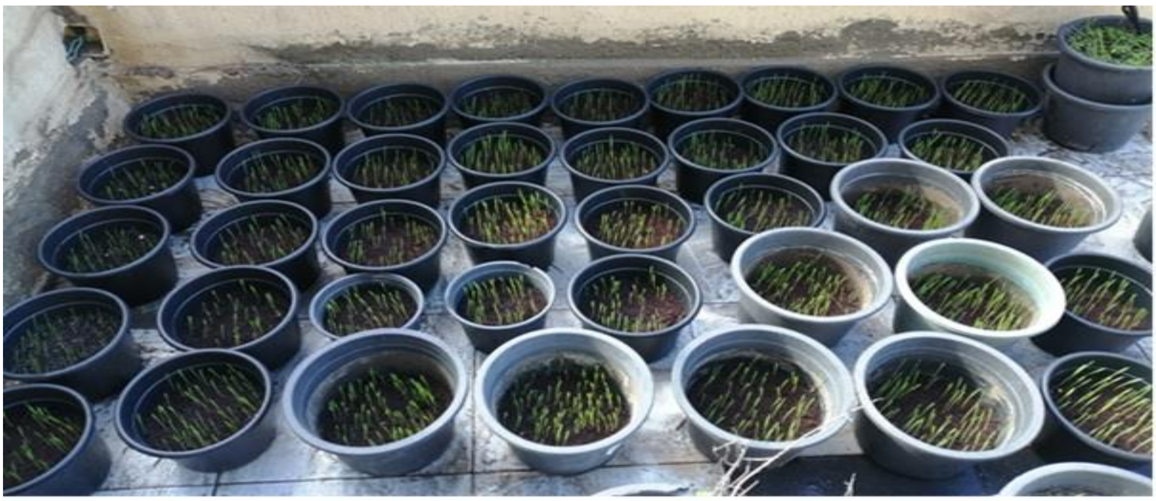

**Fig. 4:** Wheat grains germination in pots with different biochar treatments.

**Fig. 5:** Increasing wheat seedling growth with different biochar treatments after two weeks of sowing

**Cytological Studies:**

**Germination Test**:

20 healthy grains of wheat grains were grown in petri dish (12.5 cm) at the same time under room temperature. Different biochar concentrations with distilled water were added for each grain until germination (after 3 days). Alive and dead grains were measured and recorded for calculating the germination percentage (G %). Also, the germination percentage was calculated between the different treatments and concentrations **.**

**Cell Division (Mitosis):**

Mitotic studies were carried out on root tips from germinated wheat seeds. After treatments with different biochar sources for 72 hours, seeds were germinated, and the roots were collected as primary roots. Also, adventitious roots were collected from the older germinated seeds **.**

**Killing and Fixation:**

The root tips were washed with distilled water and placed in 95% ethanol and glacial acetic acid (3:1) v/v for 24 hours at room temperature for killing and fixation**.**

**Root Tip Conservation:**

Root tips were removed from the fixative solution and placed in 70% ethanol, stored in a refrigerator (4-5OC) until examined (Samad and Kabir, 1992)**.**

**Carnoy's Solution:**

In preparation for staining, root tips were removed from the 70% alcohol and treated with 1N hydrochloric acid at 60OC for 10-12 minutes for hydrolysis. The hydrolyses root tips were washed with distilled water and placed in Petri dish containing acetocarmine stain for 1 to 1.5 hours. After staining the root tips were placed on a clean slide with a drop of aceto-carmine and squashed with a rusted needle then they were covered with a cover slide **.**

**Slides Preparation and Staining:**

The whole preparation was warmed. The cover slide was pressed slightly with filter paper. Slides were examined after preparation. Karyotyping system (FUJITSU-YLCM264618-Made in Germany, 1000x) was used for taking photos of the divided and abnormal cells using the same magnification. Different mitosis stages were observed and calculated under the different treatments**.**

**Mitotic and Aberration Index** (%)**:**

It's the percentage of cells in mitosis at any time. Mitotic index (%) was measured from nearly 1000 cells (from 10 silds) for each treatment as the total of the divided cells, and the number of different mitosis stages (Prophase, Metaphase, Anaphase and Telophase). The abnormal cells were observed and detected**.**

$$MI\ (\%) = \frac{Number\ of\ divided\ cell}{Total\ number\ of\ cells}$$

$$Aberration\ (\%) = \frac{Number\ of\ abnormal\ cell}{Total\ number\ of\ divided\ cells}$$

## RESULTS AND DISCUSSION

### Biochar Characterization and Description:

The different sources of biochar used in the current study were analyzed and characterized by different methods such as Transmission Electron Microscopes (TEM), and different nano scales were used to detect the form and size of these materials. In addition, all the elements were calculated as found in Table 1.

For the first type of biochar generated from straw rice, the results showed a high percentage of carbon compared with the other elements. The sample recorded many elements such as Carbon, Oxygen, Magnesium, Silicon, Potassium, and Calcium. The second one of biochar from maize stalk includes higher elements compared with the rice straw biochar, thus continuing the following elements i.e. Carbon, Oxygen, Sodium, Magnesium, Silicon, Sulfate, Chlorine and Calcium. the highest two elements were Carbon and Silicon, respectively as found in Table 2.

Finally, for the last type of biochar from tree residues, the analysis of the elements showed an increase in Carbon, Calcium and Oxygen, respectively Compared with the other biochar types the Calcium was higher as found in Table 3.

**Table 1:** elements analyzed of the biochar generated from straw rice**.**

| Element | Peak area | Area sigma | K factor | Abs. corn. | Weight % | Weight sigma | Atomic % |
|---|---|---|---|---|---|---|---|
| C | 14249 | 1337 | 2.504 | 1.00 | 86.73 | 1.14 | 91.80 |
| O | 1539 | 79 | 1.871 | 1.00 | 7.00 | 0.66 | 5.56 |
| Mg | 359 | 41 | 1.064 | 1.00 | 0.93 | 0.13 | 0.49 |
| Si | 1357 | 67 | 1.000 | 1.00 | 3.30 | 0.31 | 1.49 |
| K | 725 | 50 | 0.970 | 1.00 | 1.71 | 0.18 | 0.56 |
| Ca | 145 | 30 | 0.953 | 1.00 | 0.34 | 0.07 | 0.11 |
| Total | 100.0 | | | | | | |

**Table 2:** Elements analyzed of the biochar generated from stalk maize**.**

| Element | Peak area | Area sigma | K factor | Abs. corn. | Weight % | Weight sigma | Atomic % |
|---|---|---|---|---|---|---|---|
| C | 68434 | 1236 | 2.504 | 1.00 | 78.36 | 0.35 | 87.86 |
| O | 6518 | 158 | 1.871 | 1.00 | 5.58 | 0.15 | 4.69 |
| Na | 397 | 84 | 1.191 | 1.00 | 0.22 | 0.05 | 0.13 |
| Mg | 420 | 79 | 1.064 | 1.00 | 0.20 | 0.04 | 0.11 |
| Si | 28606 | 288 | 1.00 | 1.00 | 13.08 | 0.22 | 6.27 |
| S | 953 | 91 | 0.959 | 1.00 | 0.42 | 0.04 | 0.18 |
| Cl | 1086 | 91 | 0.983 | 1.00 | 0.49 | 0.04 | 0.19 |
| K | 3724 | 119 | 0.970 | 1.00 | 1.65 | 0.06 | 0.57 |
| Total | 100.0 | | | | | | |

**Table 3:** Elements analyzed of the biochar generated from tree residues from farm**.**

| Element | Peak area | Area sigma | K factor | Abs. corn. | Weight % | Weight sigma | Atomic % |
|---|---|---|---|---|---|---|---|
| **C** | 117372 | 3835 | 2.504 | 1.00 | 65.37 | 0.75 | 80.86 |
| **O** | 25343 | 303 | 1.871 | 1.00 | 10.25 | 0.25 | 9.79 |
| **Mg** | 5788 | 168 | 1.064 | 1.00 | 1.37 | 0.05 | 0.84 |
| **P** | 2116 | 144 | 1.013 | 1.00 | 0.48 | 0.03 | 0.23 |
| **Cl** | 2963 | 142 | 0.983 | 1.00 | 0.65 | 0.03 | 0.27 |
| **K** | 1038 | 112 | 0.970 | 1.00 | 0.22 | 0.02 | 0.09 |
| **Ca** | 100864 | 508 | 0.953 | 1.00 | 21.37 | 0.47 | 7.92 |
| **Total** | **100.0** | | | | | | |

**Cytological Studies:**
**Mitosis Division and Aberrations:**

During the current study, different biochar levels and sources effect were tested on the cell division and mitotic index besides their effect on chromosomal aberration on wheat root tips (Table 4). The wheat root tips were collected and examined under microscopic (100x) to detect the several mitosis phases such as prophase, metaphase, anaphase, and telophase, in addition to cytokinesis. The chromosomal aberrations were observed and detected to show the effect of different biochar levels and sources such as stickiness, lagging chromosome bridge, multinuclei, ergon, abnormal prophase, abnormal metaphase, abnormal anaphase, elongation of nucleus content and gab chromosome.

Data in Table 4 showed the difference in the mitotic index under the different biochar levels and types. The highest mitotic index was recorded for the plants treated with 6% maize stalks biochar at 79.46% and the lowest mitotic index was recorded for the plants treated with tree residues biochar at 6%. On the other side, the results of aberrations were in vise versa with mitotic index, thus was 36.09% abnormal cells under 6% tree residues biochar compared with the lowest percentage was 14.62% for the cells under maize stalks biochar 3%.

**Table 4:** The effect of different biochar sources and levels on mitotic index and abnormal percentage

| Treatments | Types of biochar | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Maize Stalks Biochar | | | Rice Straw Biochar | | | Tree Residues Biochar | | |
| **Concenterations** | **1%** | **3%** | **6%** | **1%** | **3%** | **6%** | **1%** | **3%** | **6%** |
| **TOC** | 2500[a] | 2480[a] | 2502[a] | 2450[b] | 2400[b] | 2560[c] | 2500[a] | 2485[a] | 2522[a] |
| **TDC** | 1840[b] | 1950[a] | 1988[a] | 1650[b] | 1720[a] | 1580[b] | 1720[a] | 1430[b] | 1355[c] |
| **%MI** | 73.60 | 78.63 | 79.46 | 67.35 | 71.67 | 61.72 | 68.80 | 57.55 | 53.73 |
| **TAC** | 320 | 285 | 340 | 402 | 452 | 399 | 500 | 522 | 489 |
| **AP** | 17.39[a] | 14.62[b] | 17.1 [a] | 24.36[a] | 26.28 [a] | 25.3 [a] | 29.07[b] | 36.5 [a] | 36.09[a] |

* TOC: Total observed cells, TDC: Total divided cells, MI: Mitotic index, TAC: Total abnormal cells, AP: Abberation percentage

The current results detected the importance of using biochar in the agricultural domain and their effect on cell division and chromosomal aberration, besides their effect on increasing the growth hormones in plants related to growth. A similar study by (Major *et al*., 2009) reported that the variations in biochar sources and levels have been informed to alter biochar's subsequent impacts on soil quality and crop efficiency. Also, another study by Younis *et al*. (2015a-c) stated that biochar generated from the cotton stick, at four hundred and fifty (450 °C) enhanced photosynthetic pigments, chlorophyll content (a and b), carotenoids, and gas replacement attributes under Cd and Ni stress.

In addition, there are many reports by Zhang *et al*. (2014) and Hmid *et al*. (2015) presented that biochar use decreased oxidative stress in plants under trace elements (TEs) stress. For example, rice straw biochar (RSBC) reduced MDA contents in rice flag leaf. The current results are agreeing with Hmid *et al.* (2015) who showed that biochar application enhanced the enzyme activity, glutamate dehydrogenase, malic enzymes, and other parameters with the effect of Zn stress.

The current data is in a line with Racioppi *et al.* (2019) who evaluated the response of wheat to biochar from wood in Italy. The authors detected the impact of biochar soil amendment on gene expression using real-time polymerase chain reaction (RT-PCR). They determined the content of some hormones like gibberellins (GAs), auxins (IAA), and abscisic acid (ABA). Their findings demonstrated that biochar pointed an impact on the growth performances of wheat cultivars at the molecular level. They reported that biochar enhanced the germination rates. Other studies reported that in general, crop growth, germination, and yield depend on both genetic and environmental factors as reviewed by Graziano *et al.* (2019).

The balance of plant hormones is of vital significance in the germination rate. Biochar as soil amendment application can promote auxin, and gibberellin regulation, and enhance plant growth (French, and Iyer-Pascuzzi, 2018). Also, they reported that Gibberellins are the growth hormones that improved plant elongation, germination, and flowering in cereal grains. Additionally, French and Yyer-Pascuzzi (2018) reported that biochar as soil amendment supports plant growth through the motivation of the (GA) pathway. the influence of biochar on plant growth performance and on the hormones and enzyme activity implicated in plant growth has not been completely examined as reported by Farhangi-Abriz, and Torabian, (2018).

The results are agreeing with Futa *et al*. (2020) who studied the impact of different levels of biochar on enzymatic activity and soil properties at different levels for the period of 48-60-72 months after treatment. Their results showed an increase in pH, and N content in the biochar compared to the control soil. Also, the results suggested that the soil enzymatic activity has been increased under biochar application.

Other results by Copley *et al*. (2017) examined the impact of biochar on some plant diseases and the molecular responses through interaction with biochar application. Commonly, the transcript abundances of genes linked with principal metabolisms like glycolysis, tricarboxylic acid (TCA) cycle, starch, amino acid and glutathione metabolism are composed of genes linked with plant defense and immunity like salicylic acid (SA) and jasmonic acid pathways were observed after exposure of plants to a high concentration of biochar.

Other studies reported the impact of biochar in different areas such as Viger *et al*. (2015) detected the global gene expression arrays on biochar-treated plants, to identify the growth-promoting plant hormones, brassinosteroid and auxin, and their signaling molecules, as plant growth stimulation, with limited influences on genes controlling photosynthesis. Besides, promote the genes for the cell wall loosening for enhanced activity in membrane transporters for sugar, nutrients and aquaporins for well water and nutrient application and movement of sugars for metabolism in the plant.

Conclusion

The main conclusion of the current study is detecting the benefits of different biochar resources and levels on plant growth and their effect on chromosomal aberration.

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