



RESEARCH ARTICLE

Mitigating role of activated charcoal against crop residue induced soil sickness

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Abstract

Monoculture of high yielding crops is a common trend in modern agro-ecosystems. Farmers have recently embraced the practice of keeping stubbles and plant waste and incorporating them into their cropping systems. As soon as crop debris comes into touch with the soil, it begins to decompose. It is recognised that plant leftovers change the physical characteristics of soil. Crop quality and yield decline due to soil sickness brought on by the buildup of phytotoxic chemicals. The first stages of residue breakdown have been reported to have the most phytotoxicity. In the present study, the effect of *Zea mays* residue was evaluated on the succeeding crop (wheat). Soil culture experiments were conducted in the greenhouse. The residue (plant debris) was added to the garden soil in two concentrations (4,8 g/kg soil). The residue resulted in decline of growth of wheat seedling as observed through seedling height and biomass, pigment, protein and sugar content. Elevation in lipid peroxidation occurred reflecting the damage to cellular systems, even though a substantial increase in activity of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (POD) was observed. The phenolic content of soil also increased due to addition of residue. The most significant aspect of this study was to investigate the contribution of activated charcoal (AC) towards evading residue induced soil toxicity. AC has large surface area and pore volume, as well as due to its polarity possesses tremendous adsorptive capacity with complex chemical and physical properties. AC can be easily mixed into soil media and it did not impose any negative effect on the wheat seedlings and also decreased the level of soil phenolics in comparison to untreated soil. AC demonstrated counteracting effect on phenolic contents of crop residue and presented as possible solution for alleviation of the residue induced soil sickness.

Keywords: Activated charcoal; allelochemicals; antioxidants; biomass; lipid peroxidations; wheat seedlings; maize residue.

Introduction

Plant residue is described by Kumar and Goh (2000) as plants or plant parts left in the field for breakdown following crop harvest. Farmers began to retain and use plant detritus, such as stubbles, into their cropping systems from the 1970s. Retaining stubble is advantageous for enhancing soil structure. Nevertheless, as time went on, new hybrids with shorter life cycles were developed. Consequently, two to three crops are generated in a single season. Thus,

in contemporary agro-ecosystems, monoculture of highly productive crops is a prevalent tendency. However, by preserving equilibrium in crop diversity, area, and time, agro-ecosystem sustainability can be attained.

Negative implications of stubble retention should not be disregarded, even though it may improve soil structure and water infiltration. As soon as crop debris comes into touch with the soil, it begins to decompose. It is recognised that plant leftovers change the physical characteristics of soil. Crop wastes discharge a wide range of organic and inorganic substances into the soil. A build-up of phytotoxic chemicals leads to soil illness, which lowers crop quality and production. Early residue breakdown phases have been reported to have the most phytotoxicity. The primary concern with crop residue is the allelopathic nature of these substances and how they affect different or identical crop plants. According to several reports (Kato *et al.* 2005, Amist *et al.* 2015), phytotoxins from crop residues have a negative impact on the growth of seedlings of subsequent crops. The addition of residue also raised the phenolic content of the soil.

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In North India, maize, usually referred to as “corn” or “bhutta,” is a commonly farmed crop. It’s in the Poaceae family of grasses. This crop is kharif. High quantities of hydroxamic acids are found in maize. 2-b-D-glucopyranosyloxy-4-hydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA-Glc) is the primary hydroxamic acid found in maize, whereas 2-b-D-glucopyranosyloxy-4-hydroxy-1,4-benzoxazin-3-one (DIBOA-Glc) is found in smaller amounts (Tipton *et al.* 1967).

According to Cheremisinoff and Morresi (1978), activated carbon is a useful tool for allelopathy research because it can adsorb a wide range of big chemical molecules. Allelopathy studies are theoretically possible using activated carbon, as it reduces allelopathic effects while having minimal effects on nutritional dynamics (Inderjit and Nilsen, 2003). It is simple to incorporate activated carbon into soil media or even the natural environment (Prati and Bossdorf, 2004). Due to its polarity, AC has a large surface area and pore volume in addition to having complicated chemical and physical properties that make it an excellent adsorptive material. Large-scale characteristics of coal can help agricultural soils become better. It speeds up the activation of microorganisms (Warnock *et al.* 2007) and helps to lessen the acidity of the soil (Yao *et al.* 2012). Nitrogen is immobilised in part by charcoal (Lehmann *et al.* 2006). Charcoal really strengthens the physical, chemical, and biological characteristics of severely damaged tropical soils. Through its richness and composition, charcoal also affects the biological community of the soil (Liang *et al.* 2010). Greenhouse gas emissions are reduced as a result of its application in agricultural systems. Activated charcoal changes the water content of the soil, which affects plant root development and the health of the soil’s fauna. It helps the soil fix nutrients like carbon, nitrogen, phosphorus, potassium, and calcium by acting as an adsorbent compound of those nutrients (Sohi, 2012).

The current study assessed the impact of *Zea mays* residue on the crop that followed (wheat). The most significant aspect of this study was to investigate the contribution of activated charcoal (AC) towards evading residue induced soil toxicity. AC can be easily mixed into soil media and it did not impose any negative effect on the wheat seedlings and also decreased the level of soil phenolics in comparison to untreated soil. AC demonstrated counteracting effect on phenolic contents of crop residue and presented as possible solution for alleviation of the residue induced soil sickness.

Material and Methods

Experimental Design

The experimental treatments consisted of 2- factors: (i). Maize residue rates: 2 (4, 8 g/kg soil) (ii). Activated charcoal: 2 (Control, Activated charcoal). The experiment was done in glasshouse at optimum temperature (20-25°C) with natural sunlight. The maize plant residue was incorporated in soil

at 4 and 8 g/kg soil. The control was garden soil alone. Additionally, 8 g maize residue/kg soil was mixed with 5% activated charcoal (w/w) i.e. 50g/kg soil.

Soil analysis

Determination of physiochemical properties of soil

Analysis of physiochemical properties of soil was done from Soil Testing Laboratory (STL), Motilal Nehru Farmers Training Institute, IIFCO, Phulpur, Allahabad.

Determination of total phenolic content of soils

The amount of total phenolics in soil was determined using Folin-ciocalteu reagent following the method of Swain and Hillis (1959).

Plant growth measurements

Plant height of test plants was measured with the help of centimeter measuring tape. Seedlings biomass was measured on the basis of dry weight (mg). Test plants were kept in oven at 80°C. After 48h plant materials were removed from oven and dry weight was recorded.

Biochemical Assays

Sugar content

The quantification of total soluble sugars (TSS) was done following Hedge and Hofreiter (1962). Fresh leaf (100 mg) was homogenized in 5mL of 95% (v/v) ethanol. After centrifugation, 1 mL of supernatant was mixed with 4 mL anthrone reagent and heated on boiling water bath for 10 min. Absorbance was recorded at 620 nm after cooling. The amount of sugar was determined by the standard curve prepared from glucose.

Protein and pigment contents

Protein content was determined according to the method of Lowry *et al.* (1951). The amount of protein was calculated with reference to standard curve obtained from bovine serum albumin. The leaves (10 mg) were homogenized with 10 mL of 80% acetone. Chlorophylls and carotenoids were extracted and quantified following the method of Lichtenthaler (1987).

Determination of reactive oxygen species (ROS) and oxidative damage index

Electrolyte leakage

Fresh leaves were harvested from stressed and control seedlings. They were cut into small pieces. Leaves (100 mg) then immersed in 10 mL of deionized water and kept for 24 h at 10 °C. Electrical conductivity was measured and then the leaves were autoclaved for 15 min, cooled to 25°C, and the electrical conductivity was measured for the second time. Electrolyte leakage was evaluated as the percentage injury index following the formula of Sullivan (1971):

$$EL = [1 - (T_1 / T_2)] / (1 - C_1 / C_2) \times 100\%$$

Where, C_1 and C_2 : measured conductivity in control samples before and after autoclaving, respectively; T_1 and T_2 : conductivity measurements of water-stressed samples before and after autoclaving, respectively.

Lipid peroxidation

Lipid peroxidation was measured as the amount of malondialdehyde (MDA) determined by thiobarbituric acid reactive substance (TBRs) as described by Heath and Packer (1968). Fresh leaf (200 mg) was ground in 0.1 w/v trichloroacetic acid (TCA) and centrifuged at 10,000g for 10 min. One mL supernatant was mixed with 4mL of 0.5% thiobarbituric acid prepared in 20% (w/v) TCA. The mixture was then heated at 95°C for 30 min and again centrifuged after cooling. The absorbance of the supernatant was recorded at 532 nm and corrected by subtracting the non-specific absorbance at 600 nm. The MDA concentration was calculated using the extinction coefficient of 155 mM⁻¹ cm⁻¹ and expressed as nmol g⁻¹FW.

Superoxide radical

Superoxide radical (SOR) was determined according to Elstner and Heupel (1976). The fresh leaf (0.10 g) was homogenized in 65mM potassium phosphate buffer (pH7.8) and centrifuged at 10,000g for 10 min at 4°C. Assay mixture consisted of 65mM potassium phosphate buffer (pH 7.8), 10mM hydroxylamine hydrochloride and leaf extract (1ml) and was incubated for 20 min at 28°C. Thereafter, 17mM sulfanilamide and 7mM naphthyl ethylene diamine dihydrochloride (NEDD) were mixed to the incubated reaction mixture. After 15min of incubation, diethyl ether was mixed to the same reaction mixture gently and centrifuged at 2000g for 5 min. The absorbance of the colored aqueous phase was recorded at 530 nm. Standard curve prepared with NO₂⁻ was used for SOR calculation.

Hydrogen peroxide

Hydrogen peroxide (H₂O₂) was determined by Velikova *et al.* (2000). For the extraction and assay fresh samples (150mg) of plants were homogenized in 0.1% (w/v) trichloroacetic acid. The extract was centrifuged at 4°C at 10,000g for 10 min in cooling centrifuge (Remi instruments C 24). The reaction mixture (2ml) contained leaf extract, 10mM potassium phosphate buffer (pH 7.0) and 1M KI solution. Absorbance were recorded at 390 nm. H₂O₂ concentration was calculated by using a standard curve prepared with graded concentration of H₂O₂.

Assay of enzymatic antioxidants

For the extraction and assay of enzymes, fresh leaf (0.25g) was homogenised with 0.1 M sodium phosphate buffer containing 1% (w/v) polyvinyl pyrrolidone (pH 7.0) in a pre-cooled mortar and pestle. The extract was centrifuged at 4°C at 14,000g for 30 min in cooling centrifuge (Remi instruments C 24). The supernatant was used for the assay

of superoxide dismutase, catalase, ascorbate peroxidase and guaiacol peroxidase.

The activity of *superoxide dismutase* (EC 1.15.1.1) was estimated by the nitroblue tetrazolium (NBT) photochemical assay following Beyer and Fridovich (1987). The reaction mixture (4 mL) consisted of 20 mM methionine, 0.15 mM ethylene diamine-tetra acetic acid (EDTA), 0.12 mM NBT and 0.5mL supernatant. The test tubes were exposed to fluorescent lamp for 30 min and identical unilluminated assay mixture served as blank. One unit of enzyme was measured as the amount of enzyme which caused 50% inhibition of NBT reduction.

Catalase (EC1.11.1.6) activity was assayed following Cakmak and Marschner (1992). Assay mixture (2 mL) contained 25 mM potassium phosphate buffer (pH 7.0), 10 mM H₂O₂ and 0.5 mL enzyme extract. The rate of H₂O₂ decomposition for 1 min was monitored at 240 nm and calculated using extinction coefficient of 39.4 mM⁻¹cm⁻¹ and expressed as enzyme unit mg⁻¹protein. One unit of catalase was determined as the amount of enzyme required to oxidise 1μM H₂O₂ min⁻¹.

Ascorbate peroxidase (EC1.11.1.11) was assayed following Nakano and Asada (1981). Assay mixture (2mL) contained 25 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.25 mM ascorbate, 1.0 mM H₂O₂ and 0.2 mL enzyme extract. H₂O₂ was the last component to be added. The absorbance was recorded for 1 min at 290 nm using extinction coefficient of 2.8 mM⁻¹cm⁻¹. Enzyme specific activity was measured as enzyme unit mg⁻¹protein as the amount of enzyme required to oxidise 1μM H₂O₂ min⁻¹.

Guaiacol peroxidase (EC 1.11.1.7) was assayed following Hemeda and Klein (1990). The reaction mixture (2mL) contained 25 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.05% (v/v) guaiacol, 1.0 mM H₂O₂ and 0.2 mL of enzyme extract. The increase in absorbance due to oxidation of guaiacol was monitored at 470 nm. The enzyme activity was measured using extinction coefficient of 26.6 mM⁻¹cm⁻¹ and expressed as enzyme unit mg⁻¹protein.

Statistical analysis

Treatments were arranged in a randomized block design with three replications. Data were statistically analyzed using analysis of variance (ANOVA) by using SPSS (Ver.10; SPSS Inc., Chicago, IL, USA). Appropriate standard error of means (±SEM) was calculated for presentation with tables and graphs. The treatment means were analyzed by Duncan's multiple range test (DMRT) at P< 0.05.

Result

Soil analysis

Effects of crop residues on basic physiochemical properties of soil samples: The crop residue decreased soil pH and EC. Organic carbon increased. Available phosphorus

recorded in term of P_2O_5 (phosphorus pentoxide) showed graded increase in comparison to control. Exchangeable potassium amount measured in terms of K_2O (potassium oxide) increased considerably in residue amended soils with highest in maize residue (Table 1).

Effect of activated charcoal on phenolic contents of residues

The phenolic content of residue incorporated soil with and without activated charcoal was determined. Residues of maize amended soil have higher amount of phenolics as compared with control soil. Higher level of phenolics was found in the 15 days decomposed residues of rice, wheat and maize as compared with 25 days decomposed residue. Activated charcoal decreased the level of phenolics significantly. Phenolic content declined significantly in AC mixed residues of all crops (Figure 1).

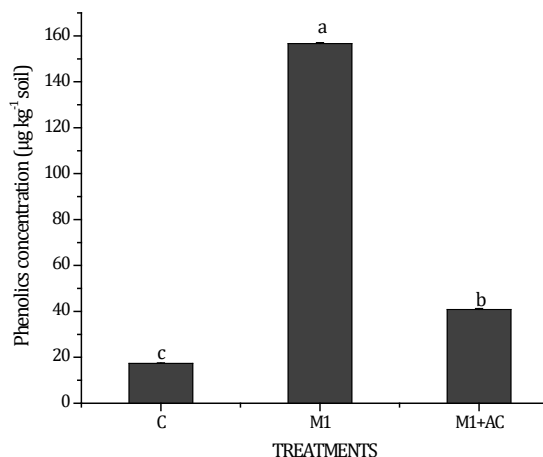
Plant height and dry weight

Maize residue affected the wheat seedlings height. Different concentrations of maize residue resulted into significant ($p < 0.05$) suppression in seedling height. Seedling height decreased by 21.43% under A_2 concentrations of maize residue. However, AC caused only 1.87% reduction in seedling height. Residue incorporated soil caused significant reduction in dry weight (DW) of seedlings. A_2 recorded maximum 24.35% decrease in DW. (Table 2). The seedling grown under controlled condition and AC treatment showed normal dry weight.

Biochemical analysis

Sugar content

Total soluble sugar content was variably affected under all stresses. Sugar content showed gradual increase under both concentrations of residue with maximum 161.9% in A_2 . However, the level decreased in AC treatment but was still higher than control (Table 2). The residue incorporation



C= control, M1= Residue amended soil, M+AC= 8 g residue kg⁻¹ soil mixed with 5% (w/w) activated charcoal

Figure 1: Phenolic content of untreated and residue treated soil and activated charcoal.

caused significant decrease in protein content. Maximum 58.93% decrease was recorded in A_2 treatment. AC treatment caused a slight increase in protein content in comparison to control (Table 2).

Pigment content

The highest amount of total chlorophyll was observed in plants under controlled condition. A gradual decrease in chlorophyll content in wheat seedlings was observed with increase in residue concentrations as highest decrease of 66.0% in A_2 treatment. Incorporation of activated charcoal elevated the chlorophyll content but was lower than control. The amount of carotenoids decreased considerably under residue treatments. Carotenoids followed the trend of chlorophyll. A_2 treatment recorded lowest amount of carotenoids. (Figure 2).

Table 1: The basic physiochemical properties of soils amended with crop residues

Soil samples	pH	Electrical conductivity	Organic carbon (%)	P_2O_5 (kg/H)	K_2O (kg/H)
Control (garden soil)	7.82	1.51	0.70	35.00	252.00
15 days decomposed maize residue amended soil	7.61	0.62	1.83	40.00	275.00

Table 2: Effects of 15 days decomposed maize residue and activated charcoal on seedling height, dry weight, sugar and protein content of *Triticum aestivum*

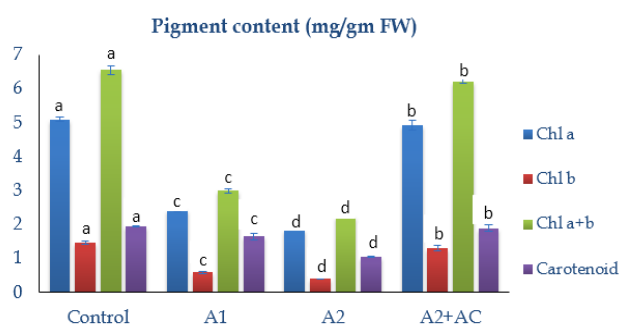
Treatments	Seedling Height (cm)	Dry weight (mg/plant)	Sugar (mg g ⁻¹ FW)	Protein (mg g ⁻¹ FW)
C	31.95 ± 0.202a	121.05 ± 0.259a	29.16 ± 0.471d	17.12 ± 0.271b
A_1	26.05 ± 0.202c	94.74 ± 0.285c	70.04 ± 1.012b	8.91 ± 0.222c
A_2	25.10 ± 0.115d	91.57 ± 0.710d	76.37 ± 1.185a	7.03 ± 0.556d
A_2 +AC	31.35 ± 0.606b	119.35 ± 0.548b	32.16 ± 0.427c	18.97 ± 0.430a

Mean±SE values followed by same letters are not significantly different at $P < 0.05$ (ANOVA and Duncan's multiple range test) $n=3$. C= control A_1 = 4 g residue kg⁻¹ soil, A_2 = 8 g residue kg⁻¹ soil, and AC= 8 g residue kg⁻¹ soil mixed with 5% (w/w) activated charcoal.

Table 3: Interactive effects of 15 days decomposed maize residue and activated charcoal on reactive oxygen species (ROS) and oxidative damage index of *Triticum aestivum*.

Treatments	SOR (nmol g ⁻¹ FW)	H ₂ O ₂ (nmol g ⁻¹ FW)	LP (nmol g ⁻¹ FW)	EL (%)
C	30.51 ± 0.752c	6.67 ± 1.443c	8.90 ± 0.328c	7.09 ± 0.120b
A ₁	46.33 ± 3.254b	26.56 ± 0.914b	22.86 ± 0.516b	30.90 ± 0.585a
A ₂	59.10 ± 1.023a	36.78 ± 0.496a	24.85 ± 0.163a	31.79 ± 0.217a
A ₂ +AC	32.21 ± 0.262c	9.27 ± 0.626c	9.025 ± 0.507c	7.71 ± 0.386b

Mean ± SE values followed by same letters within each column are not significantly different at 0.05 (ANOVA and Duncan's multiple range test) n=3. C= control, R= Residue amended soil, A₁ = 4 and A₂ = 8 g residue kg⁻¹ soil, AC= 8 g residue kg⁻¹ soil mixed with 5% (w/w) activated charcoal., SOR= Superoxide radical, H₂O₂= Hydrogen peroxide, LP= lipid peroxidation and EL= electrolyte leakage.

**Figure 2:** Interactive effects of 15 days decomposed maize residue and activated charcoal on the pigment content of *Triticum aestivum*. Mean±SE values followed by same letters are not significantly different at P<0.05 (ANOVA and Duncan's multiple range test) n=3. C= control A₁ = 4 g residue kg⁻¹ soil, A₂ = 8 g residue kg⁻¹ soil, and AC= 8 g residue kg⁻¹ soil mixed with 5% (w/w) activated charcoal.

Reactive oxygen species and index of oxidative damages

Superoxide radicals (SOR) and H₂O₂ contents were recorded highest in the plant exposed to A₂ (Table 3). Superoxide radicals (SOR) and H₂O₂ contents increased 93.6% and 451.1% exposed to A₂ over control respectively. The AC treatment decreased the level of SOR and H₂O₂ in comparison to the residue treated seedlings.

Electrolyte leakage (EL) and lipid peroxidation (LP) were taken as indicator of stress. Significant increase in EL level was recorded in the seedling in response to residue treatments. Maximum increase of 348.37% in EL was

recorded in A₂ seedlings in all residue amended soil. LP was measured in terms of MDA content which increased significantly under all the treatments. A gradual increase in MDA content was observed as result of residue incorporation with higher doses causing more elevation. Elevation of 1.34% was observed in AC. (Table 3).

Antioxidant enzyme activities

In response to 15 days maize residue, the activities of ROS scavenging enzymes showed diverse results in wheat seedlings. Activity of SOD in all treatments varied in comparison to control. A concentrations dependant increase in SOD activity was observed in seedlings grown in soil amended with 15 days old maize residue with maximum 662.5% under A₂. CAT activity was parallel to that of SOD. Lowest CAT activity was recorded in control followed by AC. Significant increase was observed in all treatments in comparison to control. The activity increased gradually in seedlings grown in residue amended soil with highest level in A₂. CAT activity decreased in in control and AC. A significant (p<0.05) effect of residue on APX and POD activities was observed. POD activity corresponded to that of APX and followed similar pattern except an impetuous 382.8% rise in A₂. The antioxidant enzyme activities were lower in AC treated seedlings (Table 4).

Discussion

Plant establishment and growth are suppressed by the release of phytotoxic chemicals into the soil by microbes during the decomposition of organic residue. Crop residue

Table 4: Interactive effects of 15 days decomposed maize residue and activated charcoal on the antioxidant enzyme activities of *Triticum aestivum*.

Treatments	SOD (EU g ⁻¹ FW)	CAT (EU g ⁻¹ FW)	APX (EU g ⁻¹ FW)	POD (EU g ⁻¹ FW)
C	11.34 ± 0.482d	0.182 ± 0.007c	0.225 ± 0.008b	0.156 ± 0.003d
A ₁	78.46 ± 0.748b	0.507 ± 0.013b	1.697 ± 0.014a	0.661 ± 0.012b
A ₂	86.49 ± 0.683a	0.550 ± 0.005a	1.832 ± 0.249a	0.754 ± 0.009a
A ₂ +AC	17.85 ± 0.190c	0.165 ± 0.003c	0.278 ± 0.003b	0.294 ± 0.012c

Mean ± SE values followed by same letters within each column are not significantly different at 0.05 (ANOVA and Duncan's multiple range test) n=3. C= control, A₁ = 4 and A₂ = 8 g residue kg⁻¹ soil, AC= 8 g residue kg⁻¹ soil mixed with 5% (w/w) activated charcoal., SOD= Superoxide dismutase, CAT= Catalase, APX= Ascorbate peroxidase and POD= Guaiacol peroxidase.

considerably decreased the rate of seed germination. Decomposing maize residue decreased respiration in tobacco seedlings and caused chlorosis and limited maize development (McCalla and Army, 1961). The early growth of other species as well as the germination of seeds were negatively impacted by the phytotoxicity of leftover maize. The weed-suppressive properties of residues and seedlings were considerably diminished when activated charcoal was added to soils, indicating a connection between phytotoxins and weed-suppression in soil. Celery residue's inhibitory impact on lettuce development is partially restored by activated carbon. (Shilling *et al.* 1992). Our results showed that seedling height decreased as a result of residue treatments. According to Vaughan and Ord (1991), phenolics are known to influence the levels of Indole acetic acid (IAA) in plants and regulate the activities of the enzymes that either synthesise or breakdown IAA. Allelochemicals have been shown to alter plant metabolic pathways, which has been linked to decreased plant development (Amist *et al.* 2015).

Dry weight significantly reduced in all treatments, with higher dosages of combination treatment being more inhibitory. Allochemicals reduced DW on a variety of plants, including *Lactuca sativa* (Hussain *et al.* 2010), soybean (EL-Rokiek *et al.* 2006), tomato (Singh *et al.* 2014) and others. Soluble carbohydrates act as stress-reduction mechanisms, preventing protein denaturation, protecting cells from oxidative stress, preventing structural and functional damage and maintaining membrane integrity. As to Asgharipour *et al.* (2011), the reason for the higher sugar content in stressed seedlings might be attributed to their reduced utilisation of sugar for growth. Previous studies have demonstrated an increase in sugar level in response to residue. The treatments had varying effects on the protein content. All crop residues: soil ratio inhibited the amount of protein. Protein content may have decreased as a result of metabolic process impairment brought on by stress and a decrease in pigment concentration. Allelopathins have been linked to a decrease in protein content, according to Venkateshwarlu *et al.* (2009). Protein deterioration results from oxidative damage caused by ROS. It's unclear if the inhibition of protein content was brought on by increased proteolysis or a decrease in de novo synthesis. The breakdown product BOA (2-Benzoxazolinone) is what causes the protein concentration in mung and cucumbers to decrease (Batish *et al.* 2006).

A decline in pigment contents in response to stresses was observed. Photosynthesis was suppressed by the action of allelochemicals in *Triticum aestivum* (Amist *et al.* 2014; Amist *et al.* 2015) and *Zea mays* (Singh *et al.* 2009). Allelopathic substances may be interfering with the synthesis of porphyrin, which is a precursor to the biosynthesis of chlorophyll (Rice, 1984). The pattern of carotenoids was

comparable to that of chlorophyll. A reduction in carotenoid content indicated the degree of oxidative stress on plants, which causes chlorophyll to degrade. The main physiological processes that are significantly impacted by allelochemicals are photosynthesis, stomatal control of CO₂ supply, thylakoid electron transport (light reaction), and carbon reduction cycle (dark reaction) (Zhou *et al.* 2006).

According to Halliwell (2006), plants that are under stress produce more ROS. Damage and the onset of oxidative stress are caused by an imbalance between the generation of reactive oxygen species (ROS) and their scavenging by antioxidant enzymes in any region of the cell. Plant cell death is caused by oxidative stress, which also causes increased membrane permeability, lipid peroxidation, damaged protein, and damaged DNA. In a range of crop plants, allelopathic stress results in oxidative burst and oxidative damage. It has been observed that the use of allelochemicals increases superoxide radicals, which can cause lipid peroxidation and membrane damage (Halliwell and Gutteridge, 1999). In present study increased electrolyte leakage (EL) was observed under residue. Allelochemicals mediated increase in EL has been reported (Amist and Singh, 2014; Amist *et al.* 2015; Yadav *et al.* 2016). Peroxidation of polyunsaturated fatty acids in biomembranes, which produces byproducts like malondialdehyde, may be the cause of damage to membrane permeability. Since lipid peroxidation is linked to oxidative damage, it is thought to be a sign of oxidative stress. Malondialdehyde (MDA) accumulation is a sign of lipid peroxidation. Wheat grown in soil supplemented with residues showed higher levels of MDA. Our findings align with the efforts of multiple researchers. Plants have higher MDA levels due to allelopathic stress (Amist *et al.* 2015). All treatments resulted in different activity of the antioxidant enzymes. Plants develop antioxidant enzymes to protect against excessive reactive oxygen species (ROS) and prevent cellular damage (Apel and Hirt, 2004). Adjusting the activity of antioxidant enzymes is a crucial way to assess how plants react to environmental stressors. The initial line of defence against ROS is made up of SODs. Cu/Zn-SOD, Mn-SOD, and Fe-SOD are SOD isoenzymes that are found in many cell organelles. They are crucial because they convert superoxide radical (O₂⁻) to H₂O₂ and O₂, lowering the likelihood that OH[•] will form by a Haber-Weiss type reaction (Gill and Tuteja, 2010). These enzymes convert superoxide radicals (O₂⁻) to H₂O₂ and O₂, reducing the likelihood of OH[•] formation. The antioxidant defense system must quickly scavenge hydrogen peroxide, the most persistent type of ROS, to release oxygen and water. According to Blokhina *et al.* (2003), it is converted to water and molecular oxygen via CAT in peroxisomes, APX in cytosol and chloroplast, and POD in cell walls. In all treatments, the patterns of CAT, APX, and POD were comparable to those of SOD. Since CAT has a lower affinity

for H_2O_2 than APX and POD and is more susceptible to superoxide radicals, APX is a more significant peroxidase that detoxifies hydrogen peroxide to water (Cakmak, 2000). In comparison to CAT, a greater induction of APX and POD was noted in the seedlings. Similar patterns to those of SOD were seen by CAT, APX, and POD in all.

In present study, activated charcoal when mixed with residue caused increase in seed germination, seedling height and dry weight. Protein and sugar contents were found to be almost equal to that of control. Lower level of EL and LP was recorded indicating plants tolerance to stress. Antioxidant enzyme activities were also lower in activated charcoal treated seedlings. The inhibitory effect of residue was associated with phenolic acids. Higher level of phenolic was found in the 15 days decomposed residue of maize. Activated charcoal decreased the level of phenolics significantly thus decreasing the toxicity of residue and indicating that residues phytotoxicity was due to phenolic acids. Activated charcoal treatments provided evidence for allelopathy when activated charcoal minimized the effect of the competitor on test species. Several studies have reported that activated charcoal mitigates the effects of allelochemicals (Inderjit and Nilsen 2003, Lau *et al.* 2008).

Conclusion

The results of the experiments in the laboratory revealed that the addition of activated carbon, significantly alleviated the allelopathy inhibition effect of maize residue. In this experiment, the addition of activated carbon resulted in growth rates that were comparable to those of the control group, indicating that activated carbon controlled the parameters governing growth and development in addition to adsorbing allelopathic chemicals. As a result, activated carbon may be able to help with the issues associated with continuous cropping. Activated Charcoal minimised the toxicity of residues, as it absorbed the phenolics from the residues incorporated in soil. This study offered empirical data or research pertaining to mitigating persistent cropping issues. Field tests will be carried out in subsequent research to find the ideal concentration in the field and look into how AC should be applied.

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