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SHORT COMMUNICATION

Effects of application of activated carbon or carbonized maize cobs on the autotoxic effects of decomposed tomato roots extracts

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ABSTRACT

We examined the effects of applied activated carbon, carbonized maize cobs and decomposed tomato roots extracts (DBE) on the growth and contents of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) activities and malonaldehyde (MDA) content in tomato roots in hydroponic culture with perlite as substrate. The high concentration DBE inhibited the growth of tomato seedlings. The DBE application, first increased the activities of SOD, POD and CAT in tomato and then decreased during the growth. The autotoxic effects were observed in tomato roots with high MDA contents. The application of activated carbon and carbonized maize cobs enhanced the negative effects, but decreased the change in enzymes activities and MDA contents.

Key words: Activated carbon, autotoxicity, carbonized maize cobs, catalase, *Lycopersicon esculentum* L., malonaldehyde, peroxidase, root residues, superoxide dismutase, tomato.

INTRODUCTION

Autotoxicity is major barrier to tomato production in continuous cropping. Autotoxic substances are released through leaching, volatilization (from shoots), root exudates and decomposed plant residues (21). The decomposed leaves are inhibitory to the seed germination and plant growth of tomato (17). The autotoxicity caused by root exudates and plant residues exists in tomato, cucumber, watermelon and melon and many autotoxic substances (such as cinnamic acid) have been isolated and identified (23). At low concentrations, the root growth is more sensitive to the autotoxic chemicals than shoot growth and seed germination (4,6). Besides, the autotoxicity increases with the production of peroxides and active oxygen species in root tips (7,24). Thus the indices of root oxidative stress (including the activity of SOD, POD, CAT and MDA content) were measured to determine the mechanism of autotoxins action and membrane lipid peroxidation (22).

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Autotoxicity can be overcome by application of beneficial microbes, organic manures and grafting (3,8,12). The organic manures are commonly used as simple and effective method (15). Activated carbon and carbonized maize cobs are economical and easily available materials. This study aimed to determine the autotoxic effects of DBE and its alleviation by activated carbon and carbonized maize cobs in hydroponic culture with perlite as substrate. It also aimed to better understand the mechanism and theoretical basis to overcome the autotoxicity.

MATERIALS AND METHODS

Tomato plants were grown in a growth chamber at 27 °C for 5 months and then roots were collected during the fruiting stage in September 2007. After drying, the roots were cut into 1-2 cm pieces and ground in Wiley Mill. The powdered material was sieved through 18-mesh sieve. Five hundred g powdered material was mixed with 500g soil and 1000 ml water. The mixture was kept in 2000 ml beakers and sealed for decomposition at 25°C for 30 days. The mixture with decomposed roots was extracted with water (1:20) for 18 h on shaker and mixed with 1/2 Hoagland nutrient solution (1:1) and thus decomposed biomass extract (DBE) was ready for future use.

Experiment was conducted in pots in plastic tunnel and in lab during April to June, 2008. Hybrid tomato 'Liaoyuanduoli' was sown in pot. At 4-leaf stage, 4 seedlings were transplanted per plastic pot (10 cm dia) with perlite as substrate. The treatments were: A. DBE (Decomposed biomass extract); B. DBE + 5% activated carbon (added into substrate); C. DBE + 5% carbonized maize cobs (added into substrate) and D. Control irrigated with 1/4 Hoagland nutrient solution only. In each treatment, there were 10 plants and arranged randomly with three replications.

Bioassay

Agar solution (1.6%, w/v) was autoclaved for 25 min at 125°C and then equilibrated in a 50 °C water bath along with a similar flask of DBE and one flask of sterile deionized water. The warm DBE was diluted appropriately with warm deionized water and then mixed in 1:1 ratio with warm agar solution, to give the desired final extract concentrations. About 10 mL extract-agar or water-agar (control) were poured into Petri dishes (9 cm dia). Twenty-five seeds were imbibed in distilled water for 12 h and placed onto the agar surface with DBE concentrations of 0.0 (distilled water), 2.5, 5,10, 25 and 50 %. The plates were covered, the edges were sealed with parafilm and placed into a growth chamber (24 °C during the 14-h light period and 22 °C during the dark period). Plates were illuminated with 350 µmol $m^{-2}sec^{-1}$ photosynthetically active radiation (PAR) of incandescent and fluorescent lamps. In each Petri dish, root and shoot lengths of all seedlings were measured at 120 h after the transfer of seeds on agar. All experiments had four replications (5). The inhibition or stimulation (%) was calculated using the following equation.

$$\text{Inhibition (-) or stimulation (+) percentage (\%)} = \left(\frac{Extracts - Control}{Control} \right) \times 100$$

Preparation of root samples: Tomato roots were sampled on 2, 5, 10 and 20 days after transplanting. Roots were collected, immediately frozen with liquid nitrogen and stored in fridge for further enzyme analysis. Crude antioxidant enzyme solutions were extracted as per previous study (10). 0.5 g root was taken and ground in 1 mL 50 mM phosphate buffer (pH 7.8) with liquid nitrogen. After adding 3 mL phosphate buffer, the ground roots were centrifuged (4000 rpm) at 4°C for 20 min and the supernatant was used to determine the enzyme activities with Beckman UV/Visible light Spectrophotometer.

Enzyme analysis:

SOD (Dismutase) activity: SOD solution was prepared using modified Marklund method (25). Crude extract was added to 4.5 mL reaction solution containing 100 mM Tris-HCl buffer (pH 8.2), 1 mM EDTA·2Na and 4.5 mM pyrogallol-HCl solution. Then absorbance was measured at 325 nm at start and 1 min later. One unit of SOD activity was defined as 0.01 change of absorbance per minute. SOD activity was expressed as U mg^{-1} protein.

POD (Peroxidase) activity measurement: 1 mL crude extract was added in 4 mL reaction medium containing 200 mM phosphate buffer (pH 6.0), 19 µl guaiacol (100%) and 28 µl H_2O_2 (30%). The absorbance was measured at 420 nm within 5 min (11). Activity of POD was expressed as µmol H_2O_2 $min^{-1}mg^{-1}$ protein.

CAT (Catalase) measurement: CAT solution was made by adding 0.2 ml crude extract in 3 ml reaction solution including 200 mM phosphate buffer, 100 mM H_2O_2. Absorbance was measured after 4 min H_2O_2 consumption at 240 nm (1). CAT activity was in µmol H_2O_2 $min^{-1}mg^{-1}$ protein.

MDA (Malonaldehyde) measurement: Total 4 mL solution was prepared by adding 1.5 mL crude extract into a mixture containing 20% trichloroacetic acid solution and 0.5% thiobarbitutric acid to determine The MDA content. The above-mentioned solution was boiled for 30 min and immediately cooled down and then centrifuged at 1800 ×*g* for 10 min. The supernatant were used for MDA measurement at 532 nm and 600 nm, respectively (9). The MDA content was calculated by correcting for compounds derived from MDA-TBA action and was expressed in nmol g^{-1} FW (fresh weight) as per following formula:

$$MDA = \frac{(D_{532}-D_{600})*V}{1.55*10^{-1}*m}$$

Where, $1.55*10^{-1}$: Molar extinction coefficient of MDA, V : Solution volume (ml), m: Root weight in crude extract (g)

Data processing: Data were processed with Excel and analyzed with SPSS11.5 software for ANOVA.

RESULTS

Bioassay on autotoxicity of DBE

The various DBE concentrations significantly decreased the radicle length over the control (Fig.1). All concentrations were inhibitory and inhibition increased with higher concentrations. The shoot inhibition was observed at 25% DBE. The DBE was less inhibitory to shoots, indicating the lower sensitivity to DBE compared to radicle. Thus tomato seedlings radicles were more sensitive to DBE than shoots.

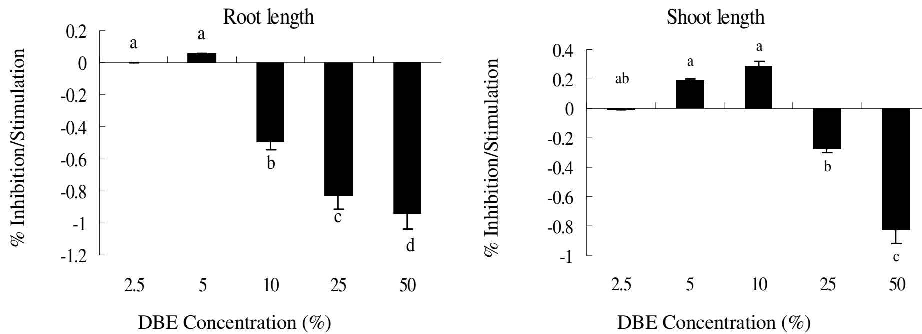

Figure 1. Effects of DBE (Decomposing Biomass extract) concentrations on the root and hypocoyl length of tomato seedlings in 5 days old crop. Values followed by the same letters are not significantly different.

POD (Peroxidase) activity

The applied DBE significantly influenced the POD activity (Fig. 2). Maximum POD activity (4.5 times of that in control) appeared in treatment A on the 5th day and then decreased gradually to the same level of that in control on the 20th day. Maximum POD value, which was almost doubled compared with control was found in treatment B (DBE + activated carbon) and C (DBE + cabonised maize cobs) on the 10th day and also decreased to the same level of that on the 20th day.

SOD (Dismutase) activity

Instead of increasing steadily as that in control, SOD activity hit the maximum value on the 5th day, which was 141% in treatment A, 90% in B and 108% in C compared with control (Fig. 2). Subsequently, SOD activity in A decreased to level on the 20th day lower than that in control. Less decrease of SOD activity was found in B and C due to the buffer effects of activated carbon and carbonized maize cobs and on the 20th day SOD activity dropped to the level as of control.

CAT (Catalase) activity

When compared with control, less CAT activities, but the same trend were found in B and C (Fig. 2). CAT activity in A reached the greater level with the maximum

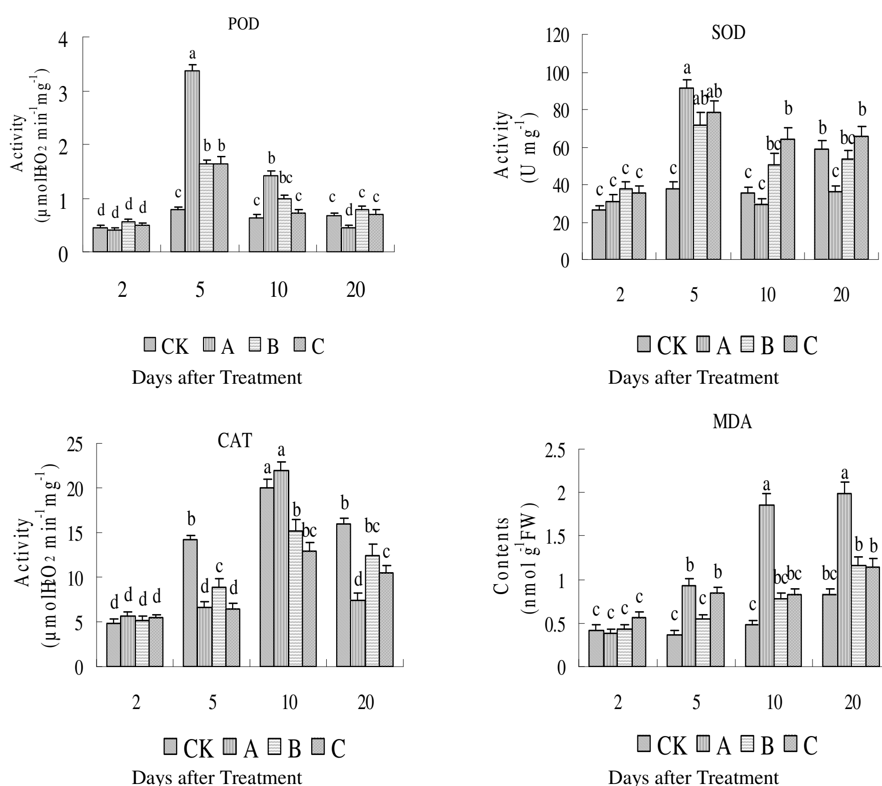

Figure 2. Effects of DBE (Decomposing Biomass extract) concentrations on the activities of enzymes and MDA content. Values followed by the same letters are not significantly different. CK:Control, A:DS, B: DS + Activated carbon , C: DS +Carbonized maize cobs

value on the 10th day and followed by rapid decrease (53%) of that in control on the 20th day. The presence of activated carbon and carbonized maize cobs caused less decrease than DBE treatment.

MDA (Malonaldehyde) content

MDA content in control roots changed little and remained at low level (Fig. 2). Compared with control, MDA content increased significantly in roots treated with DBE**.** The longer the treatment was, the more the MDA content increase. MDA content increased 3.9 and 2.4 times, respectively on the 10th day and 20th day. With addition of activated carbon and carbonized maize cobs, MDA content in B and C were lowered when compared to A, but still a bit higher (1.6 and 1.4 times) than that in control on the 10th and 20th day.

DISCUSSION

Allelopathic effects decreases the germination and plant growth of crops (2,13,20). The high concentration of DBE stunted the tomato seedlings growth, but lower concentration had less effect. Application of DBE increased the production of peroxides and active oxygen species in root tips. The defensive enzyme system (POD, SOD, CAT, etc.) plays important role in dynamic equilibrium between the active oxygen species and the removal of intracellular active oxygen (formed during the plant growth). SOD may catalyse the superoxide anion ($O_2^{\bullet -}$) removal and H_2O_2 generation. Then H_2O_2 was transformed into H_2O and O_2 by the catalysis of POD and CAT (16,18,19,21).

Day 2: The activities of antioxidant enzymes and MDA content did not change than control. It indicated that defensive system of plant reduces the free radicals at the beginning of stress.

Day 5: The antioxidant enzyme system was activated. The increase in SOD activities may be owing to the increase in $O_2^{\bullet -}$ content, when the plant was exposed to DBE. H_2O_2 accumulation resulted from the increase in POD activity. However, the CAT activity in DBE treatments did not change than control. Application of activated carbon and carbonized maize cob considerably decreased the change in POD activities than DBE treatment. The MDA contents increased in all treatments especially in DBE treatments due to the membrane lipid peroxidation.

Day 10: The application of DBE decreased the SOD and POD activity in all treatments except control, probably owing to increase in $O_2^{\bullet -}$. The decrease in POD activity caused the accumulation of H_2O_2 , which increased the CAT activity. The MDA content was still higher due to root peroxidation with reduction in POD and SOD activities.

Day 20: The POD and CAT activities decreased due to DBE severe oxidative stress and degradation of antioxidant mechanisms. The MDA content was still growing. The addition of activated carbon and carbonized maize cob were less effective over the control. The accumulation of activated oxygen species decreased the activity of enzymes, which increased the $O_2^{\bullet -}$ formation than its removal. This caused more damage to plant, which resulted in more inactivation of enzymes (14). The application of activated carbon and carbonized maize cobs to the substrate also caused similar slight changes. The enzyme activities on 20th day were similar to control. The strong adsorptive capacity of activated carbon and carbonized maize cobs decreased the allelopathic substances concentration resulting in less damage to roots. With passage of time, the inhibitory effects decreased as compared to CK. MDA content was closely related to lipid peroxidation intensity and extent of damage in plasma membranes. MDA content in our experiment coincided with the results of defensive enzymes. DBE caused more lipid peroxidation in roots and the addition of activated carbon and carbonized maize cobs increased the protective effects on plasma membranes which were seen in lower content of MDA.

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