

## Autotoxicity effects of soils continuously cropped with tomato

SUN YAN-YAN<sup>1</sup>, JIANG GUI-YING, WEI XIAO-CHUN<sup>2</sup> and LIU JIAN-GUO<sup>\*</sup>

Agriculture Department, Shihezi University, Shihezi, Xinjiang-832003, China.

E. Mail: ljg\_2010@yahoo.com

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### ABSTRACT

A pot experiment was done to determine the effects of continuous cropping and application of activated charcoal in soil on the growth, antioxidant enzyme activity and physiological parameters of photosynthesis in tomato (*Lycopersicon esculentum* Miller). The yield and biomass of tomato were significantly inhibited with the continuous cropping. The 2, 3 and 4-years of continuous cropping inhibitory the yield and biomass of tomato by 5.34%, 21.5%, 29.3% and 11.7%, 16.3%, 38.8%. The increasing duration of continuous cropping, decreased the activity of superoxide dismutase (SOD), root vigour and chlorophyll content in tomato plants. However, the peroxidase (POD) activity declined after increasing, but malonyldialdehyde (MDA) content was increased. Application of activated charcoal in soil greatly improved the physiological characteristics (SOD activity and chlorophyll content), but MDA content was decreased in tomato plants grown in soil mixed with activated charcoal than in soil without activated charcoal. Besides, the net photosynthetic rate (Pn), stomatal conductance (Gs) and transpiration rate (Tr) decreased with prolonged cropping time (4-years) to 58.7%, 45.8% and 55.1% than control (1-year), respectively. The addition of activated charcoal to soil, increased the Pn, Gs and Tr than their control (no activated charcoal), respectively by 236.9%, 106.6% and 161.9%. The results suggested that adding of activated charcoal to soil changed the autotoxic effects of tomato, the antioxidant enzymes activities and decreased the physiological parameters.

**Key words:** Activated carbon, autotoxicity, bioassay, *Lycopersicon esculentum*, physiological characteristics, tomato

### INTRODUCTION

The problem of continuous cropping is common world wide, especially in legumes, vegetables and maize. Autotoxicity is the main reason of continuous cropping problem (14,20). Autotoxicity is a special kind of allelopathy, which occurs within the same species due to release of autotoxins that adversely affects the growth of adjacent plants or subsequent plants (19,25,26). There are many studies on various aspects of autotoxicity e.g. the effects of crop residues autotoxins on seedlings or the adverse effects of decomposing substances of straw and root exudates on crop growth (6,11,21). These autotoxicity effects primarily inhibits the growth of plant roots and also shoots of plants, decreasing the photosynthesis and POD and SOD activities (major protective enzymes in protective system), resulting in problem of continuous cropping (8). The methods to overcome the adverse effects of autotoxins in soil-less system of plant cultivation are (i). use of available

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<sup>\*</sup>Correspondence author, <sup>1</sup>Forestry Administration of Shexian, Handan, Hebei-056400, China; <sup>2</sup>Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing-100081, China

microbial and (ii). crop rotation systems for upland paddy (9). However, the quickest method is the application of active carbon, which removes the autotoxins by absorption (24) or supplying the micronutrients to soil (2,11,27), or nutrients solution to promote the physiological activity of plants.

Xinjiang is favourable for tomato production, due to abundance of light and heat. Tomato is major crop in 90% China, but autotoxicity is major problem (26). Qin identified 3-organic acids (phthalic acid, benzoic acid and cinnamic acid) from tomato plant aqueous extracts and these suppressed the seed germination and caused allelochemicals accumulation (10,15). It was also discovered that not only root exudates but also the plant extracts have autotoxicity effects (28). The past studies lacked soil, therefore, it is necessary (i). to study the effects of continuous cropping on tomato growth, (ii). to find the theoretical basis to solve the problem of continuous cropping in tomato and (iii). to assess the effects of adding activated charcoal in soil on autotoxicity effects of continuous cropping.

## MATERIAL AND METHODS

### I. Plant sampling and preparation

The processed tomato variety 'Legel 87-5' was grown in clay pots (40 cm dia × 50 cm high) filled with 15 kg soil. The soil was continuous cropped for 1 year [1], 2 years [2], 3 years [3], 4 years [4] and amended control with activated charcoal (Soil : Active carbon = 500:1), which were 2 years + active carbon [2+C], 3 years+ active carbon [3+C] and 4 years + active carbon [4+C], in fields of our Experiment Farm, Xinjiang, China (45°19' N, 86°03' E) in 2007. Three seeds were sown per pot on 25 April 2007, with 6-replications and pots were arranged in a completely randomized. At seedling (June 7), flowering (June 30) and fruiting (July 16) stages, the selected plants were taken to laboratory in ice box.

### II. Methods

**Physiological measurements :** Leaf gas exchange was assessed using a Li-6400 photosynthesis system (Li-Cor, Lincoln, NE, USA). All measurements were taken between 10:00 and 12:30 h Australian EST. 2000  $\mu\text{mol m}^{-2}$ . The following settings were used for gas exchange: Reference  $\text{CO}_2$  at 400  $\mu\text{mol mol}^{-1}$  using a  $\text{CO}_2$  mixer, light intensity at 2000  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  using the Li-Cor red-blue LED light source, and leaf chamber block temperature of 35°C (ca. mean ambient air temperature at midday). System flow rate of the Li-6400 was adjusted to maintain a VPD (leaf temperature-based) of 1.2 to 3.1 kPa. Based on the photosynthetic light response curve for Sicala  $V_2$  measured in the field at the Australian Cotton Re-search Institute (13), 2000  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  PFD, corresponding to the maximum natural PFD, could saturate their photosynthetic rates. Water use efficiency (WUE) was calculated as the ratio of net photosynthetic rate to transpiration rate ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1} \text{CO}_2/\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1} \text{H}_2\text{O}$ ).

**Enzyme extraction and assay:** 0.5 g leaf and root tissue were placed in a mortar and watered with 5.0 ml phosphate buffer (pH=7.8), milling to be slurry, followed by centrifugation at 10 000 × g for 10 min at 4°C, the supernatant was used for both enzyme

assays. Superoxide dismutase (SOD) activity was measured as per Madamanchi (16). Crude extract was added to a reaction solution (3 ml) containing 50 mM phosphate buffer ( $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ ) at pH 7.8, 0.1 mM EDTA, 13 mM methionine, 2  $\mu\text{M}$  riboflavin and 75  $\mu\text{M}$  1-(4,5-dimethylthiazol-2-yl)-3,5-diphenyl-formazan (MTT). The reaction was started by exposing the mixture to cool white fluorescent light (Hitachi Co., Japan) at photosynthetic photon flux of  $50 \mu\text{mol m}^{-2} \text{s}^{-1}$  for 15 min. Then the light was switched off, the tubes were stirred and the blue colour was measured at 560 nm. Guaiacol peroxidase (POD) activity was assayed in a reaction solution (3 ml) containing 50mM phosphate buffer at pH 7.0, 1% guaiacol, 0.4%  $\text{H}_2\text{O}_2$  and 10  $\mu\text{L}$  crude extract. Increase in the absorbance due to oxidation of guaiacol was measured at 420 nm (29).

**Determination of malonyldialdehyde (MDA) content:** Lipid peroxidation was determined in terms of 2-thiobarbituric acid (TBA) reactive metabolite, chiefly malonyldialdehyde (MDA). Tomato leaves were extracted with 50 mmol/LPBS (pH 7.0). After centrifugation at  $2000 \times g$ , 0.3 ml supernatant was added to 3 ml 20% TCA (including 1%TBA). The solution was quickly cooled after water-heating at  $90^\circ\text{C}$ . Following centrifugation at  $10000 \times g$  for 10 min, the absorbance of the supernatant was measured at 532 nm. The level of lipid peroxidation is expressed as  $\mu\text{mol}$  of MDA formed using an extinction coefficient of 155 mmol/cm (18).

**Assay of root vigour:** The vigour of roots was estimated by method of Arnon (2). 0.5 g root tissue were placed in a beaker, and watered with equivalent mixture 10 ml (TTC solution and phosphoric acid buffer), root were fully immersed in equivalent mixture preserved for 3 h at  $37^\circ\text{C}$ , then watered with vitriol 2 ml (1 mol/L) to stop the reaction. At the same time make a blank experiment, (after addition of root samples, water with sulphuric acid, other operating as same as above). The roots were taken out, ground together with ethyl acetate (3-4ml) to put forward the red extract, then it was removed to the tube, and ethyl acetate was added to make the total volume of 10 ml, followed by measurement at 485 nm, with blank test, check the standard curve, you can calculate the amount of tetrazolium reduction.

**Determination of chlorophyll content:** Chlorophyll meter [SPAD-502, soil and plant analysis development (SPAD), Minolta Camera Co. Osaka, Japan] (7) was used for chlorophyll measurement on ten top fully expanded leaves (index leaves) per plot at flowering (June 30), and three SPAD readings (dimensionless values, 650/940 nm wave lengths transmittance ratio) were taken around the midpoint of each leaf blade, 30 mm apart from one side of the midrib. Thirty SPAD readings were averaged to represent the mean SPAD readings of each plot.

**Statistical analysis:** The data were analyzed using Microsoft Office Excel 2003 and SAS 6.12 programs. Means, least significant differences (LSD) of 5% level were calculated using SAS software (Vol.6.12).

## RESULTS AND DISCUSSION

### Tomato growth

The growth of shoot and of root plant parts was significantly decreased by the continuous cropping (Table 1). With the prolongation of continuous cropping, these development indices were decreased than 1-year cropping. The 2, 3 and 4-years of continuous cropping decreased the dry weight of roots to 11.0%, 47.0% and 65.9%, plant height was reduced by 17.4%, 26.2% and 36.4%, respectively. The dry weight of roots declined to 42.9% under 4-years cropping. The ratio of root/shoot was decreased to 12.8%, 14.5% and 35.5%, however, yield and biomass were slightly improved. The yield and biomass in 2, 3 and 4-years of continuous cropping was 0.95, 0.78, 0.71 and 0.88, 0.84, 0.61 times lower, respectively, than 1 year cropping. Application of active carbon in soil increased the plant height, stem diameter, dry weight (shoot and root) and the ratio of root/shoot and biomass than their control (without charcoal). The ratio of root/shoot vary significantly and was increased to 56.3%, 36.3% and 4.6% over control. Figure 1 showed the inhibitory/stimulatory effects of longer continuous cropping on tomato growth. The 2, 3 and 4-years of continuous cropping inhibitory the plant height, stem diameter, shoot dry weight and biomass of tomato by 17.4%, 26.2%, 40.9%, 4.5%, 18.8%, 27.1%, 16.1%, 47.3%, 58.6 and 11.7%, 16.3%, 38.8%, respectively, over 1 year cropping. As the treatment continuous cropping years increased (1 years to 4 years), inhibitory effects of longer continuous cropping of tomato on the growth were increased. Thus the toxins from continuous cropping plants adversely effects the tomato roots growth. Similar trends are observed in cotton, pea and cucumber, the addition of active carbon into nutrient solution decreased the allelopathic substances content in rhizosphere to promote the plant growth (3,24,26,27). The continuous cropping of plants is closely related to the allelopathic effects of autotoxins (23). For example, root secretion is important source of allelopathic substances (5,16) and the autotoxins released through root secretion and the decomposing crop residues adversely affects the plant growth through decreased absorption of hydronium (24) and water (4).

### Metabolism of internal active oxygen

#### POD activity

POD is important oxidoreductase enzymes to remove the active oxygen species from foliage cells, whose function is to prevent the cell membrane damage by superoxide anion (12). Throughout the tomato growth and development period, there was difference between the leaves and roots POD activity, the activity of root POD was higher than leaves, the trends of first increase and then decrease shows single peak trend (Figure 2). Compared with control, root POD activity was increased 32.4% at 3-years of continuous cropping, while, reduced 26.5% at 4-years of continuous cropping. After treatment with activated charcoal, the activity of POD was declined than control under 2-years and 3-years cropping, but increased under 4-years. POD activities of leaves were higher (8.3%) than their control, while, root POD activities was increased by 14.8%. Toxins from the plants of continuous cropping stimulate the POD activity and then decreased it through accumulation of toxic substances (23).

Table 1. The effects of continuous cropping on tomato growth

Treatments	Plant height (cm)	Stem diameter (cm)	Shoot (g DW)	Roots (g DW)	Root/shoot ratio	Biomass (g/m <sup>2</sup> )	Yield (kg per plant)
Continuous cropping (1-year)	34.39a	2.88a	8.58b	1.19a	0.20c	43.39b	1.81a
Continuous cropping (2-years)	28.41c	2.75ab	7.20c	1.04b	0.17d	38.32c	1.71ab
Continuous cropping (3-years)	25.38d	2.34c	4.52e	1.18a	0.16d	36.32c	1.42c
Continuous cropping (4-years)	20.34e	2.10d	3.55g	0.66d	0.13e	26.57e	1.28d
Continuous cropping (2-years +C)	31.83ab	2.77ab	9.13a	1.01b	0.30b	46.85a	1.78a
Continuous cropping (3-years + AC)	31.28b	2.63b	4.79d	0.91c	0.30b	41.34b	1.65b
Continuous cropping (4-years + AC)	21.86ab	2.33c	4.05f	0.69fd	0.35a	29.34d	1.48c

AC: Activated Charcoal, DW: Dry weight, Biomass: include shoot and root. Data are the mean of 3 replicates with standards errors. Within columns, treatments with the same letter are not significantly different by LSD at the 5% level.

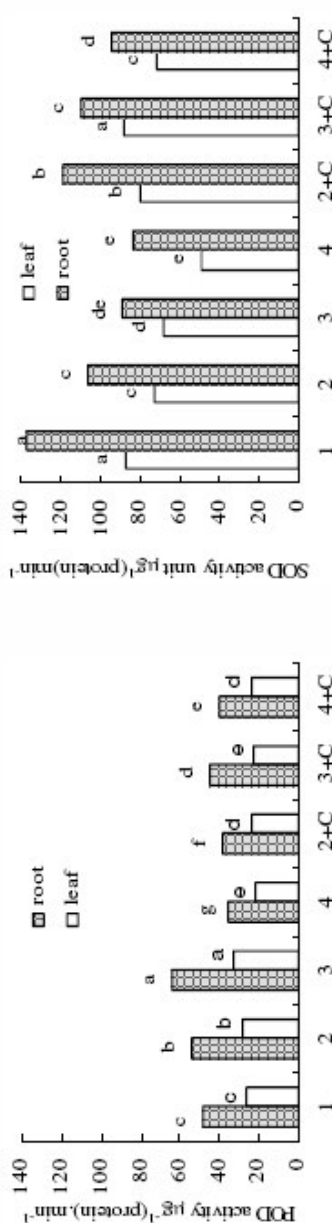


Figure 2. POD and SOD activity in the leaves and roots of tomato under different years of continuous cropping. 1,2,3,4 = 1,2,3 and 4 years of continuous tomato cropping, 2+C, 3+C and 4+C = 2,3 and 4 years of continuous tomato cropping + activated charcoal.

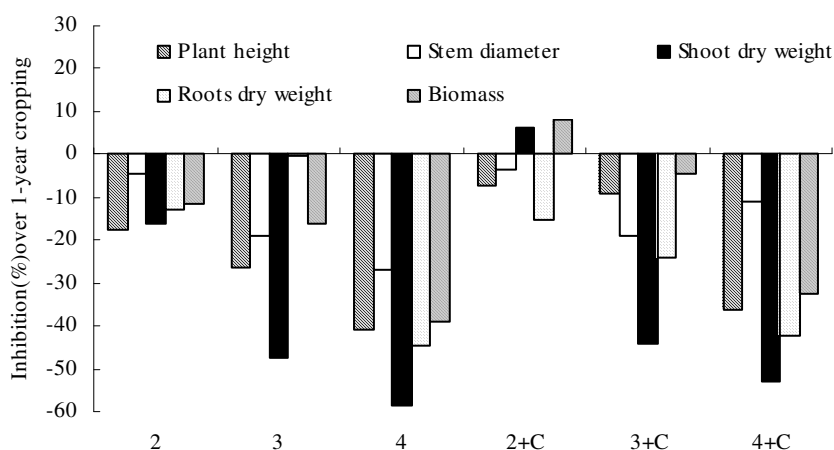


Figure 1. Inhibition effects of longer continuous cropping on tomato growth. 1,2,3,4 = 1,2,3 and 4 years of continuous tomato cropping, 2+C, 3+C and 4+C = 2,3 and 4 years of continuous tomato cropping + activated charcoal.

### SOD activity

Superoxide dismutase enzyme (SOD) have the specific function to remove the superoxide anions of biological body, balancing the oxygen free radicals, then protecting plant cells. The accumulation of toxic substances decreased the activity of SOD, so the greatest damage was after 4-years cropping. The inhibitory effects were more on roots than on leaves (Figure 2). The activity of SOD decreased with the increase in period of continuous cropping years. SOD activity of leaves after 1-year cropping was increased to 16.5%, 21.7% and 43.6% compared to 2, 3 and 4 years continuous cropping (Figure 2), whereas the activity of root SOD was increased by 22.4%, 34.7% and 35.2%. Activated charcoal treatment increased the activity of SOD. After 2, 3, 4-years continuous cropping, the SOD activity of leaves increased up to 9.4%, 29.2% and 45.7% and that of roots increase up to 11.2%, 22.6% and 6.2% respectively, compared with their control.

### MDA content in tomato leaves

With the increase in time of continuous cropping (Figure 3), the content of MDA increased by 29.8%, 34.9% and 54.6% under continuous cropping for 2, 3 and 4 years, respectively, compare with 1-year cropping. After application of activated charcoal to soil, the content of MDA was declined by 0.2%, 17.1% and 26.8% respectively under 2, 3 and 4 years. This indicated that due to accumulation of autotoxins, the influence of autotoxins on structure and function of plasma membranes were enhanced with increased duration of continuous cropping. The root secretions of watermelon changes the activity of antioxidant enzymes (POD activity was declined), more active oxygen species accumulated leading to lipid peroxidation of plasma membranes in roots, which declined the yields (29).

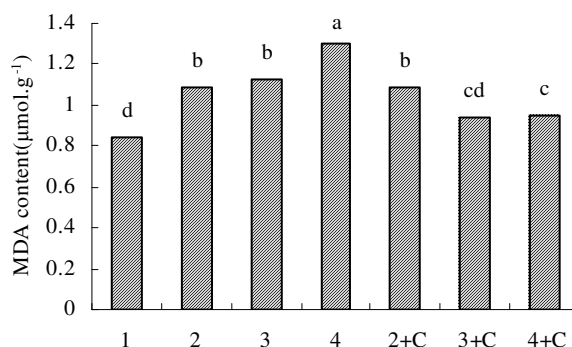


Figure 3. MDA content in tomato leaves under different years of continuous cropping. 1,2,3,4 = 1,2,3 and 4 years of continuous tomato cropping, 2+C, 3+C and 4+C = 2,3 and 4 years of continuous tomato cropping + activated charcoal.

#### Tomato root vigour

The Tomato root vigour was inhibited in continuous cropping soil and the degree of inhibition increased with increasing years of continuous cropping. It was decreased by 23.0%, 36.8% and 53.4% respectively, after 2, 3 and 4 years continuous cropping, compare to 1 year continuous cropping (Figure 4). After adding active carbon in soil, root vigour was increased to 14.5%, 25.2% and 39.2% respectively, than control.

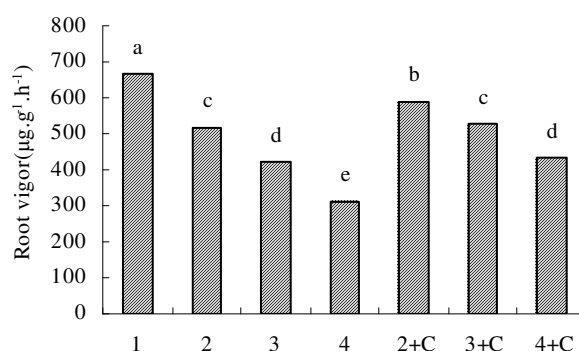


Figure 4. Tomato roots vigor under different years of continuous cropping. 1,2,3,4 = 1,2,3 and 4 years of continuous tomato cropping, 2+C, 3+C and 4+C = 2,3 and 4 years of continuous tomato cropping + activated charcoal.

#### Chlorophyll content

Plant leaves absorb the light energy, assimilate carbon dioxide and water and produce organic matter by photosynthesis. The function of photosynthetic pigment from chlorophyll was to catch and fix the sun energy, which shows photosynthetic efficiency (Figure 5). With prolonged time of continuous cropping, the contents of chlorophyll were reduced to 73.6 mg·g<sup>-1</sup>, 66.9 mg·g<sup>-1</sup>, 56.85 mg·g<sup>-1</sup> and 47.13 mg·g<sup>-1</sup> respectively, under 1-4

years continuous cropping. The maximum reduction in chlorophyll occurred after 4-years continuous cropping and it was 36.0% lower than after 1-year cropping. After adding the activated charcoal into soil, the content of chlorophyll increased by 27.1% and 53.4% respectively, than control after 3 and 4 years cropping.

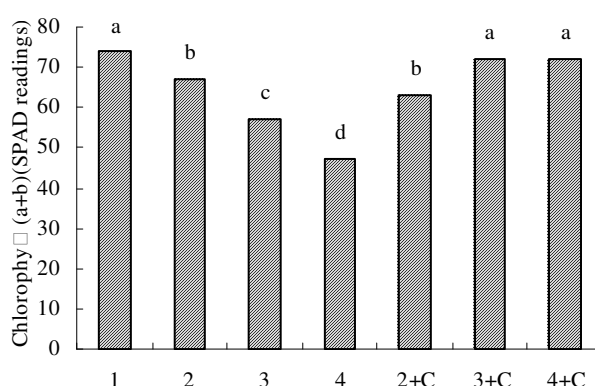


Figure 5. Chlorophyll content in tomato leaves under different years of continuous cropping. 1,2,3,4 = 1,2,3 and 4 years of continuous tomato cropping, 2+C, 3+C and 4+C = 2,3 and 4 years of continuous tomato cropping + activated charcoal.

Table 2. Effects of continuous cropping on tomato net photosynthetic rate and gas exchange parameters

Treatment	Net photosynthetic rate (Pn)	Stomatal conductance (Gs)	Intercellular CO <sub>2</sub> conc. (Ci)	Transpiration rate (Tr)	Water use efficiency (Wue)
Continuous cropping (1-year)	3.948c	0.037c	168.56d	1.963c	2.011c
Continuous cropping (2-years)	3.948c	0.032d	167.69d	1.866c	1.913d
Continuous cropping (3-years)	3.570d	0.020e	207.31b	1.226e	1.884e
Continuous cropping (4-years)	2.310f	0.020e	218.42a	0.882f	1.848f
Continuous cropping (2-years+AC)	1.630g	0.029d	180.50c	1.704d	1.736g
Continuous cropping (3-years+AC)	2.959e	0.054a	118.07f	2.849a	2.590a
Continuous cropping (4-years+AC)	7.379a	0.042b	140.09e	2.310b	2.378b

AC: Activated Charcoal; Data are the mean of 3 replicates with standards errors. Within columns, treatments with the same letter are not significantly different by LSD at the 5% level.

### Net photosynthetic rate and gas exchange parameters

With longer time of continuous cropping of tomato, Pn, Gs, Tr and Wue were reduced, but intercellular Ci was increased (Table 2). After adding activated charcoal into soil, Pn, Gs, Tr and Wue were enhanced under 3 and 4-years. Pn increased by 219.4% and 236.9% respectively, compare to their control, Gs increased by 169.7% and 106.6%, Tr were 2.32 and 2.62 times, and Wue were 1.37 and 1.29 times, but Ci were significantly declined to 28.7% and 35.9%. This shows that autotoxic substances were produced gradually from plants with the time of continuous cropping and then disordering the metabolism of physiology and closing stomas, so blocking diffusion of CO<sub>2</sub>, leading to suppression of photosynthesis. These results are similar to Wu *et al.* (22) and Baziramakenga *et al.* (3) reporting that some physiological indices of cucumber (Root

vigour, net photosynthetic rate and yields etc.) in continuous cropping declined remarkably.

Based on our research, we can fully support the activated charcoal role for autotoxicity, since more intense autotoxic effects occur in continuous cropping of tomato, and demonstrated that autotoxicity is one of the main reasons of problem of this system. Further work is needed to study the chemical nature of allelopathic substances, and the effects of different components on the growth and development, the modes of production and release, mechanism of transfer and accumulation to find the methods to eliminate the problem of continuous cropping.

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