0971-4693/94 US $ 5.00
Allelopathy Journal 23 (2): 437-444 (2009) *International Allelopathy Foundation* 2009
Table: -, Figs : 4

Effects of applied phthalic acid and phloroglucionol dihydrate on the root oxidative damage in tomato seedlings

WEN-BO ZHANG, SHU-HONG ZHANG, EN-PING ZHANG*, LIANG-LIANG LI and TIAN-LAI LI

College of Horticulture
Shenyang Agricultural University, Shenyang 110161, China.
E. Mail: zhangep024@163.com

(Received in revised form: February 12, 2008)

ABSTRACT

We examined the effects of main autotoxic substances (phthalic acid and phloroglucionl dihydrate, separated in our previous study), on root oxidative damage of tomato seedlings. Changes in superoxide dismutase (SOD, EC 1.15.1.1), peroxidase (POD, EC 1.11.1.7), catalase (CAT, EC 1.11.1.6) and malondialdehyde (MDA) and their activities in roots were measured. Potted tomato seedlings were cultured in perlite and treated with phthalic acid (PA) and phloroglucinol dihydrate (PD) as exogenous autotoxins at 1 mM, 5 mM and 10 mM concentrations. The application of both PD and PA, and especially PD increased the MDA contents. The activities of SOD, CAT and POD depended on autotoxins (PA or PD), their time of action and concentration. The enzyme activities increased with application of PA on 5th day and decreased on 10th day except at 10 mM PA. On 20th day, the activities of all enzymes decreased except SOD at 1 mM. Similar trend of enzyme changes was presented in the treatments of PD, except POD activity that kept growing on the 10th day. Results indicated the adverse effects of exogenous PA and PD on enzymes of antioxidant defence system, resulting in lipid peroxidation in roots of tomato seedlings.

Key words: Autotoxins, catalase, enzymes, lipid peroxidation, malondialdehyde, peroxidase, phlorogucial dehydrate, phthalic acid, superoxide dimutase, tomato seedlings.

INTRODUCTION

Autotoxicity exists both in natural and agricultural ecosystem, the later have attracted increasingly scientist's attention (14). It is a process in which a species or its decomposing residues release phytotoxins into the environment to inhibit germination and growth on the same species (17,18). The detrimental effects of autotoxicity (plant population decline, regeneration failure and subsequent yield declines) occurs in rice (8), tomato (12), cucumber (22), tea (19), horsetail (4) and Chinese fir (11).

The autotoxicity related soil problems in tomato were reported six decades ago (9). The aqueous extracts of tomato leaves inhibits the seedlings growth and biomass accumulation in tomato (12). Potential autotoxicity also exists in root exudates of hydroponic tomato (15). Roots in soil directly comes in close contacts with autotoxic substances released from the plants during their growth, therefore, it is the key organ for

*Correspondence author

exploring autotoxicity. Plants release the allelochemicals as mixture, rather than a single compound. The effects of individual constituents are often different than mixtures, in which the synergic and antagonistic effects occur (1). However, the mechanism of action of individual constituents in tomato seedlings is not known. In previous research, PA and PD had been isolated and identified in the root exudates of tomato (13). Therefore exogenous PA and PD were applied to determine their oxidative damage in tomato seedlings. To investigate the mechanism between exogenous autotoxins and membrane lipid peroxidation, we measured root index of oxidative stress (activities of SOD, POD, CAT) and damage degree (MDA content).

MATERIALS AND METHODS

Based on our previous study, PA and PD chemical were applied as autotoxic substances. The chemicals were first dissolved in methanol and then in distilled water. Series concentrations (1, 5, and 10 mM) of each chemical solution were prepared.

Pot Culture

The experiments were conducted from March 4 to May 20, 2008. Hybrid tomato 'Liaoyuanduoli' was used to find the oxidative damage. At 4-leaf stage, tomato seedlings were transplanted in the hydroponic system with perlite as substrate and irrigated with 50% Hoagland nutrient solution with or without the chemicals. Control seedlings were irrigated with 80 ml nutrient solution together with 80 ml water every two days. PA or PD were applied at 3 (1,5,10 mM) conc. and Distilled water as control. Each treatment with 15 potted plants was arranged randomly and the pots were rotated daily for uniform sunlight.

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 (12). 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 was centrifuged (4 000 rpm) at 4^{o}C for 20 min and the supernatant was used to determine the enzyme activities with Beckman UV/Visible light Spectrophotometer.

Enzyme analysis: SOD solution was prepared using modified Marklund method (23). 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. SOD activity was expressed as µmol/min/mg protein.

For POD activity measurement, 1 ml crude extract was added in 4 ml reaction medium containing 200 mM phosphate buffer (pH 6.0), 19 ul guaiacol(100%)and 28 ul H_2O_2(30%). The absorbance was measured at 420 nm within 5 min (16). Activity of POD was expressed as µmol/min/mg protein.

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/min/mg protein.

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 for MDA testing. 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 (10). The MDA content was calculated by correcting for compounds derived from MDA-TBA action and was expressed in nmol/gFW (fresh weight) as per following formula:

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

Where, $1.55*10^{-1}$: Extinction coefficient of MDA

RESULTS AND DISCUSSION

SOD Activity

Both PA and PD had both inhibitory and stimulatory effects on SOD activities. Positive effect was observed at low concentration (1 mM), but with the increase of concentrations to higher level (5 mM or 10 mM) SOD activities increased (on 5th and 10th day) and decreased finally on 20th day (Fig. 1). In the roots of seedlings treated with 1 mM PA and PD, the SOD activities increased 1.8 and 2 times than control 5 days after the treatment, respectively. 10 days later the stimulatory effects attenuated but still existed. On 20th day the SOD activity rebounded especially for PA treatment. The same trend in SOD activity was found in 1mM and 5 mM treatments with PA and PD, in which activity peaks appeared at 5th day. The activity value of 10 mM treatment continued to increase till 10 days after the application. The stimulatory effects then decreased and finally reversed to inhibitory effect on the 20th day for 5 mM and 10 mM treatments. PD showed more inhibitory effects on SOD activities at the highest concentration than PA

POD Activity

The PA and PD increased the POD activity over control at 2, 5 and 10 days after treatment. The POD activity peak appeared on the 5th day for PA and on the 10th day for PD. For both substances, sharp decrease in POD activity occured 20 days after treatments for all concentrations. PA proved more inhibitory than PD due to more resistance of POD to PD action (Fig. 2). The uniform response to the three concentrations of PA and PD led to the conclusion that POD was more dependent on time of their action than on concentration.

CAT Activity

The PA and PD treatments had no significant effects on CAT activities on 2nd and 5th day after treatment (Fig. 3). The activity of CAT was reduced on 10th day in all treatments, except at 10 mM PA. In control, the CAT activity in roots of tomato seedlings increased with time. This increasing trend of enzyme activity was very slight when plants were treated with PA, but the increasing range varied with PA and PD, respectively. On 20th day, the CAT of PA treatments were sharply decreased simultaneously with an increase in PD . There were no significant effects of all concentrations of PD on CAT

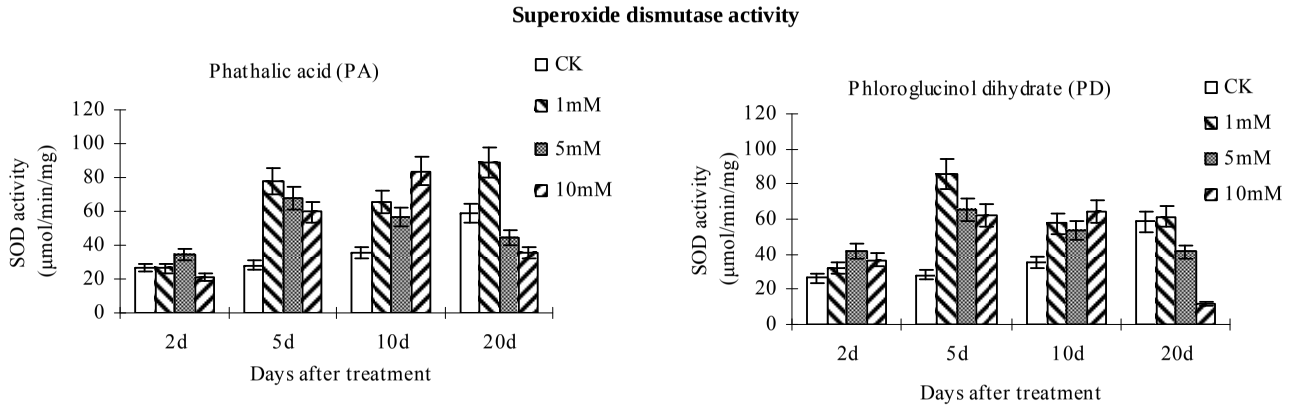

Figure 1. Effects of different concentrations of PA and PD on superoxide dismutase activity in tomato seedling roots.

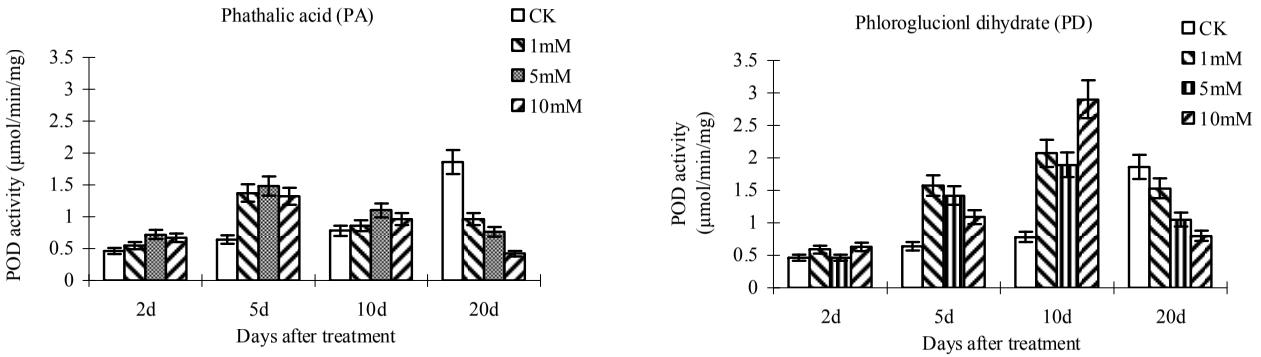

Figure 2. Effects of different concentrations of PA and PD on peroxidase activity in tomato seedling roots

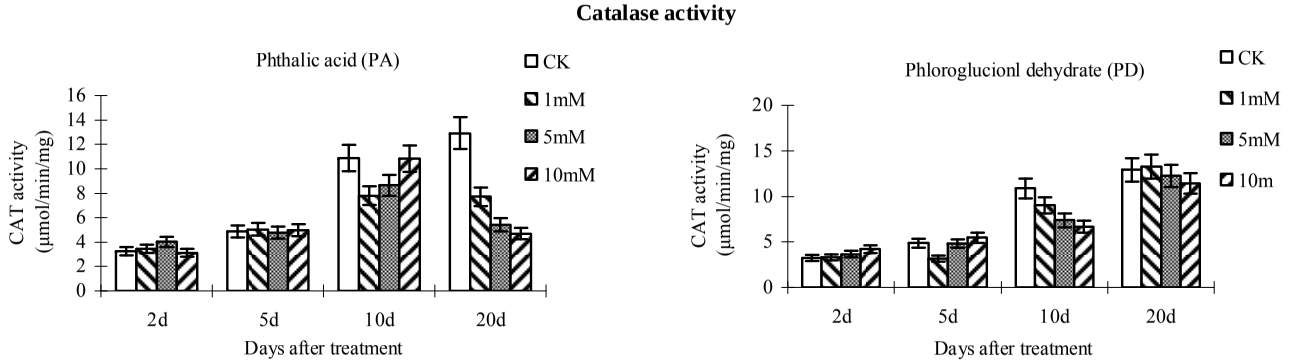

Figure 3. Effects of different concentrations of PA and PD on catalase activity in tomato seedling roots.

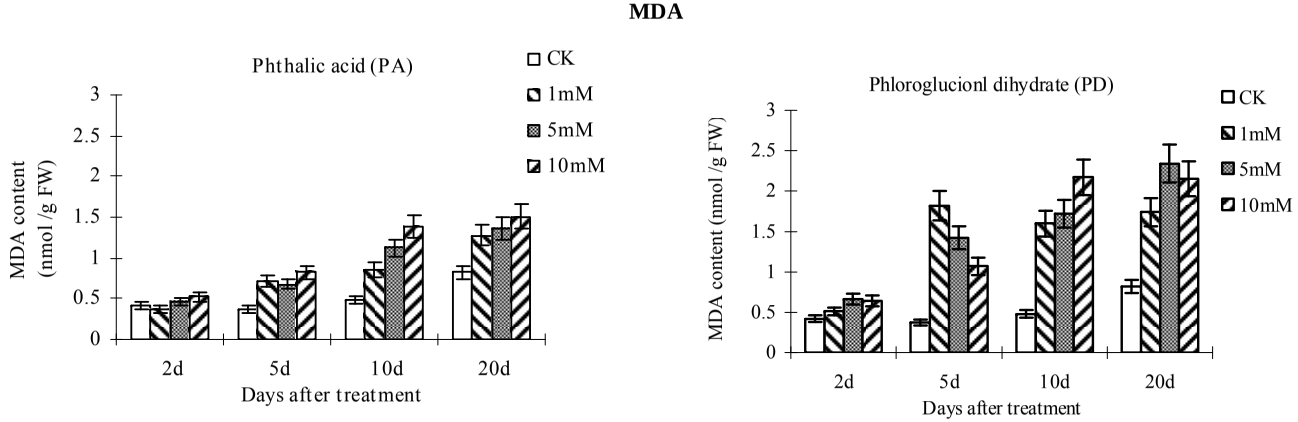

Figure 4. Effects of different concentrations of PA and PD on MDA content in tomato seedling roots.

activity except on 10^{th} day and at 1mM PD on 5^{th} day, where CAT activity was decreased (Fig. 3). However, the PA had a stronger effects on CAT activity with time and the increase of concentration.

MDA Content

The oxidative effects of PA and PD on the lipid degradation were indicated by MDA contents (Fig. 4). The application of PA and PD increased the MDA contents. The longer the roots were treated and the higher was the concentration, the more MDA content was found. Significant stimulatory effects of PA and PD on MDA contents were observed compared to control. On 5^{th} day, the MDA contents in PA treatments were 91% (1 mM), 81% (5 mM), 121% (10 mM) and it increased to 392% (1 mM), 284% (5 mM), 189% (10 mM), respectively for PD treatments. MDA accumulated with prolonged duration and increase in concentration. PD showed stronger oxidative effects than PA, leading to peroxidation of membrane lipids in roots of tomato seedlings.

Poor germination rate and plant growth under allelochemical stresses were observed in tomato seedlings in previous studies (7,13). Application of PA and PD considerably increased the production of peroxides and active oxygen species (AOS) in allelochemical-treated root tips (21) and forced the plant to oversynthesise O_2^- and H_2O_2 followed by perturbations of antioxidant enzymes system. The antioxidant enzymes and MDA content did not change over to control on the 2^{nd} day. It indicated that defencive system of the plant prevailed due to slight overloading and the free radicals were alleviated by scavengers due to their relative short endurance.

Furthermore (on 5^{th} day), the synthesis of free radicals predominated over the defensive quality of system, the antioxidant enzyme system was activated and the activity increased. Similar findings have been already reported (3, 20). The SOD activity was stimulated due to decrease in the content of O_2, when the plant is exposed to PA and PD at low concentration and the harmful damage is lessened (27). The same results were also obtained with other allelochemicals (6). Formula 1 showed the process of O_2^- removal and H_2O_2 generation by SOD catalysis. Then H_2O_2 was transformed into H_2O and O_2 (Formula 2 and Formula 3).

(i) $2O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$ (Formula 1)

(ii) $2H_2O_2 \xrightarrow{CAT} 2H_2O + O_2$ (Formula 2)

$AH_2 + H_2O_2 \xrightarrow{POD} A + H_2O$ (Formula 3)

POD activity greatly increased due to H_2O_2 accumulation. The CAT activity in PA treatments did not change much than control, but decreased when treated with 1mM and 5 mM PD. Thereby the membrane lipid peroxidation MDA contents increased in all treatments especially in 1 mM and 5 mM PD treatments. The MDA increases, when a plant is subjected to various stress conditions (5).

On the 10^{th} day of the application of PA, the SOD activity decreased in 1 mM and 5 mM treatments, probably owing to increase in O_2^-. POD activity in these treatments decreased causing H_2O_2 accumulation. The CAT activity of those treatments was also lower than control. Hence MDA content was promoted with application of 1 mM and 5 mM PA. Although the application of 10 mM PA improved the SOD activity, but with the

reduction of POD and CAT the MDA content was still high, resulting from the root peroxidation. Effects of PD on SOD and CAT activity were the same as PA, but POD activity differed. This can be presumably explained that POD activity is independent of the application of PD.

In the end of treating (on 20th day), the allelochemicals caused deep oxidative stress in target tissues and degraded the antioxidant mechanisms (2). Although the SOD and POD activities in 1 mM PA rebounded to some extent, damage of O_2^- was not counteracted and the increase of MDA level was still observed. Besides, the activity of SOD and POD in 5 mM and 10 mM PA were drastically reduced during this period leading to the more MDA. On the other hand, 1 mM PD increased the SOD activity, but decrease POD and CAT activities causing oversynthesis of H_2O_2. The application of 5 mM and 10 mM PD decreased the antioxidant enzyme activities triggering the peroxidation in membrane lipid of tomato roots. The PD had more effects on roots of tomato seedlings than PA accounting for the higher content of MDA.

ACKNOWLEDGEMENTS

This study was financially supported by the Liaoning Scientific and Technological Program (2006215001) and Shenyang Agricultural University Supervisor Programme (2006).

REFERENCES

1. Aebi, H. (1984). Catalase. In: *Methods in Enzymology* (Ed., L. Paker). Volume **105:** 121-126. Academic Press, Orlando, USA.
2. Aenavoli, M.R., Cacco, G., Sorgona, A., Marabottini, R., Paolacci, A.R., Ciaffi, M. and Badiani, M. (2006). The inhibitory effects of coumarin on the germination of durum wheat (*Triticum turgidum* ssp. *durum*, cv. Simeto) seeds. *Journal of Chemical Ecology* **32**: 489-506.
3. Bais, H.P., Vepachedu, R., Gilroy, S., Callaway, R.M and Vivanco, J.M. (2003). Allelopathy and exotic plant invasion: From molecules and genes to species interactions. *Science* **301**: 1377-1380.
4. Batish, D.R., Singh, H.P. and Kohli, R.K. (2001).Vegetation exclusion under *Casuarina equisetifolia* L: Does allelopathy play a role? *Community Ecology* **2**: 93-100.
5. Baziramakenga, R., Leroux, G.D. and Simard, R.R. (1995). Effects of benzonic and cinnamic acids on membrane permeability of soybean roots. *Journal of Chemical Ecology* **21**: 1271-1285.
6. Chou, C.H. and Lin, H.J. (1976). Autotoxication mechanism of *Oryza sativa*. I. Phytotoxic effects of decomposing rice residues in soil. *Journal of Chemical Ecology* **2**: 353-367.
7. Einhellig, F.A. (2004). Mode of allelochemical action of phenolic compounds. In: *Allelolpathy: Chemistry and Mode of Action of Allelochemicals* (Eds., F.A. Macias, J.C.G. Galindo, J.M.G. Molinilo and H.G. Cutler). pp. 217-238. CRC Press, Boca Raton, FL, USA.
8. Fageria, N.K. and Baligar, V.C. (2003). Upland rice and allelopathy. *Communications in Soil Science and Plant Analysis* **34**: 1311-1329.
9. Hirano, T. (1940). Studies on soil sickness of tomatoes. *Japanese Journal of Soil Science and Plant Nutrition* **14**: 521-530.
10. Hodges, D.M., Delong, J.M., Forney, C.F. and Prange R.K. (1999). Improvig the thiobarbituric acid-reactive-substance assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta* **201**: 604-611
11. Huang, Z.Q., Haig, T., Wang, S.L. and Han, S.J. (2002). Autotoxicity of Chinese fir on seed germination and seedling growth. *Allelopathy Journal* **9:** 187-193.
12. Kim, Y.S. (1987). A bioassay on susceptibility of selected specier to phytotoxic substances from potato plant. *Korean Journal of Botany* **30**:59-68

13. Kim, Y.S. and Kil, B.S. (1989). Identification and growth inhibition of phytotoxic substances from tomato plants. *Korean Journal of Botany* **32**: 41-49.
14. Liu, Y.H., Zeng, R.S., Chen, S., Liu, D.L., Luo, S.M., Wu, H. and An, M. (2007). Plant autotoxicity research in southern China. *Allelopathy Journal* **19**: 61-74.
15. Loffredo, E. and Senesi, N. (2006). Allelochemical activity of root exudates from horticultural plants hydroponically grown and the regulation role of humic substance. *18th World Congress of Soil Science*. Philadelphia, Pennsylvania, USA.
16. Maehly, A.C. and Chance, B. (1954). The assay of catalase and peroxidase. *Methods of Biochemical Analysis* **1**: 357-359.
17. Miller, D.A. (1983). Allelopathy in forage crop systems. *Agronomy Journal* **88**: 854-859.
18. Singh, H.P., Batish, D.R. and Kohli, R.K. (1999). Autotoxicity: Concept, organisms and ecological significance. *Critical Review in Plant Science* **18**: 757-772.
19. Wang, X.P. (1990). Current status and control approaches of land degradation of tea plantation. In: *Land Degradation in China*, (Ed., X.P. Wang). China Science Press, Beijing. pp. 355-361.
20. Xiao, C.L., Zheng, J.H., Zou, L.Y., Sun, Y., Zhou, Y.H. and Yu, J.Q. (2006). Autotoxic effects of root exudates of soybean. *Allelopathy Journal* **18**:121-128.
21. Yang, G.Q., Wan, F.H., Liu W.X. and Zeng, X.W. (2006). Physiological effects of allelochemicals from leachates of *Ageratina adenophora* (Spreng.) on rice seedlings. *Allelopathy Journal* **18**: 237-246..
22. Yu, J.Q., Shou, S.Y., Qian, Y.R., Zhu, Z.J. and Hu, W.H. (2000). Autotoxic potential of cucurbit crops. *Plant and Soil* **223**: 147-151.
23. Yuan, Y. H., Luo, S.J., Yu, W., Sun, L. J. and Zhao, F. M. (2003). *National Standard of People's Republic of China* : Ministry of Health, China committee of Standards, Beijing, China. pp: 413-414.(In Chinese)