https://doi.org/10.26651/allelo.j/2018-44-1-1148 0971-4693/94 Euro 20.00
Allelopathy Journal 44 (1): 01-12 (May, 2018) *International Allelopathy Foundation* 2018
Table: 2, Figs : 6

Replant problem and soil toxicity of passion fruit (*Passiflora edulis* Sims) in China

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(Received in revised form: March 18, 2018)

ABSTRACT

We investigated the soil toxicity in passion fruit plantations, the effects of old soils on the growth of the replanted passion and changes in photosynthetic parameters, physiological and biochemical parameters in the passion fruit leaves. In laboratory bioassays, we found that older soils of 1-, 2- and 3-year plantations significantly inhibited the root length of lettuce and passion fruit. The growth parameters (stem girth and perimeter, vine length, leaf area and shoot dry weight of passion fruit seedlings were significantly decreased when cultured in the older soils, compared with the virgin soils. Photosynthesis in the replanted seedling leaves cultured in the older soils were significantly lower than that cultured in the unplanted soils. The activity of antioxidant enzymes [superoxide dismutase (SOD), Peroxidase (POD) and catalase (CAT)] were significantly decreased in the replanted seedling leaves, along with decrease in insoluble carbohydrate and soluble protein. In replanted seedling leaves, the level of malondialdehyde (MDA) and abscisic acid (ABA) significantly increased, but the levels of indole-3-acetic acid (IAA) and cytokinins (ZR) of significantly decreased. Thus the older soils of passion fruit plantations have negative effects on the replanted passion fruit seedlings, suggesting the presence of soil autotoxicity in passion fruit plantations.

Keywords: Antioxidant enzymes, autotoxicity, endogenous hormones, *Passiflora edulis* Sims, passion fruit, photosynthetic parameters, plantations, replant, soil, soil toxicity

INTRODUCTION

Passion fruit (*Passiflora edulis* Sims.) is native of tropical America and is popular due to its aroma, nutritional and medicinal properties (1,15). There are two types of passion fruit species in China, (i). yellow passion fruit (*P. flavicarpa*) and (ii). purple passion fruit (*P. edulis*) (Fig.1). Passion fruit is a perennial. In southern China, passion fruit yield is higher in the first year and later gradually declines in the second and third years. To overcome this problem, farmers usually remove the passion fruit plants in December and replant the new passion fruit seedlings in old fields in next March i.e. after 4- months. However, this does not significantly improve the growth and yield of passion fruit. The use of different fertilizers does not solve this problem (2,19). Hence, new planting needs to be done on the soil not previously growing passion Fruit. The difficulty in raising new plantation on older soils, suggested that these soils may be a cause of the replant problem.

Soil toxicity has been widely reported in many agriculture crops [cucumber (6,23), peanut (18), strawberry (4,14), grape (11), bean (3), asparagus (9,21), eggplant (7) etc.]. However, there is no report of replant problem and soil toxicity in passion fruit cultivation.

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Hence, this study aimed to investigate the soil toxicity in passion fruit plantations and the effects of older soils on the growth and photosynthetic, physiological and biochemical parameters in the passion fruit leaves.

Figure 1. The photographs of purple passion

MATERIALS AND METHODS

The experiment was done from March to November 2016, at the purple Passion fruit plantation (*P. edulis*) in Xinluo town, Longyan City, Fujian Province, China. Elevation: 600 - 700 m above sea level, Latitude: 25°0′ N and Longitude: 116°57′ E. Annual rainfall: 1700 mm, relative humidity: 80% and annual average temperature: 16-20 °C. The physicochemical properties of soils are given in Table 1.

Table1. The general physiological and chemical properties of the soils of consecutively cultured passion fruit plantations

Soil age	pH	Total N ($g\ kg^{-1}$)	Total P ($g\ kg^{-1}$)	Total K ($g\ kg^{-1}$)	Available N ($mg\ kg^{-1}$)	Available P ($mg\ kg^{-1}$)	Available K ($mg\ kg^{-1}$)
0	5.89	2.37	1.56	1.54	25.42	78.45	280.06
1	5.23	2.25	1.39	1.62	26.17	78.82	289.37
2	4.57	2.42	1.51	1.65	27.39	82.37	307.53
3	4.16	2.16	1.48	1.58	28.39	85.26	315.17

Soil sampling and soil toxicity assessment

The top soil leaves and litter were removed and soil samples were collected upto 5-20 cm depth. The rhizosphere soils were collected from 1-, 2- and 3-years old purple passion fruit (*P. edulis*) plantations in Xinluo town of Longyan City, Fujian Province, China. After the passion fruit harvest ended in December 2015, 12 plants from each age field were chosen randomly and dug the roots up to 40 cm depth. The soils attached to the roots were collected as the rhizosphere soils. Soil samples from the area without passion fruits were collected and used as blank control (0-age).

Soil toxicity was measured by soil sandwich bioassay method (10) with slight modification. In brief, 30 mL of agar (0.8 %) was dissolved in hot water, cooled to 45 °C and added into a 9 cm Petri dishes containing 15 g fresh soils from each age group or blank control soil and mixed well. After solidification, 2 mL of agar (0.5 %) solution was added to the surface of soil-agar layer. After cooling, 10 pre-geminated seeds of lettuce (*Lactuca sativa*) and purple passion fruit (*P. edulis*) were placed on the agar culture medium. There were six replicates for each treatment. The plates were incubated in an artificial climate chamber [25 °C with 12 h (7: 00-19: 00) of 2400 Lux light intensity]. The root lengths of lettuce and passion fruit were measured after 3 days and 10 days, respectively. The relative percent inhibition [1(%)] was calculated as under:

$$I\,(\%) = (1 - \text{Experimental sample/Blank control}) \times 100\ \%.$$

Replanting of Passion fruit seedlings

For re-planting 50 m^2 area (length 10 m and width 5 m) in each of 1-, 2- and 3-years age of purple passion fruit (*P. edulis*) plantation was selected, and 50 m^2 area (never planted earlier with passion fruit) was served as control. The experiment was done from March to November 2016. The seedlings were raised from 5-cm long cuttings, purchased from a farmer and transplanted in pits (90 cm dia) on April 12, 2016. The parameters of young seedling, were determined at 30 days after Transplanting of cuttings in Pits. Passion fruit seedlings about 5 cm height were planted in square pits of 90 cm (depth 45 cm) in April 2016. One Kg organic fertilizer Yousubao® (purchased from Guangxi Hongshengyuan Environmental Protection Technology Co., LTD of Guangxi, China, ingredient list: Organic matter ≥ 45% and total nutrients ($N + P_2O_5 + K_2O$) ≥ 5.0%) and 0.3 g urea was spread evenly at the bottom of pit, then covered with 5 cm soil. One Passion fruit seedling was planted per pit and a wooden stick was inserted into the pit to support plant vine. In each test sites including control site, 50 seedlings were planted. The field was irrigated every 3-days to maintain the soil moisture. Thirty days after culture, the plant growth parameters and physiological and photosynthetic parameters of passion fruit and leaves were determined as under.

Measurement of growth parameters of passion fruit seedlings

(i). **Stem girth and perimeter:** Twenty plants were randomly selected from each experimental. We transplanted plants in 50 m^2 area of each experimental site with 1-, 2- and 3-years age soils and the control soil, (described in ‘Replanting of Passion fruit seedlings’). to determine stem girth, vine length, leaf area and shoot dry weight and photosynthesis. The stem diameter at the branching of root and stem of vine (about 1 cm high) was measured using a vernier caliper and the stem perimeter of per plant was determined using the formula:

$$C = \pi \times D;$$

Where, C: Stem perimeter; $\pi = 3.14$; D: Diameter.

(ii). **Vine length:** Twenty plants were randomly selected from each experimental site. The length from the branch point of the root and to the end of the vine was measured using a ruler.

(iii). **Leaf area:** Twenty plants were randomly selected from each experimental site. The length and width of the second internode leaf of each plant were measured using a ruler. The leaf area was calculated according to Zhang and Wang (24) using the following formula:

Area of one leaf = length × width × 0.7,

(iv). **Shoot dry weight:** This was determined after the measurement of photosynthetic parameters. Twenty plants were randomly selected from each expmental site. The above ground parts of plants were cut at the branch of root and stem of the vine. These parts were oven-dried at 110°C for 60 min and at 80°C for 48 h to a constant weight,

Photosynthetic parameters of passion fruit leaves

Twenty plants were randomly selected from each experimental site. The second internode leaf of each plant was used for measurement of photosynthetic parameters. A LI-6400XT Portable Photosynthesis System (Li-Cor, Lincoln, NE, USA) was used to measure the net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration and transpiration rate. Chlorophyll content was measured using a SPAD-502 PLUS (Konica Minolta Camera Co. Ltd., Japan). These parameters were measured between 9.00 a.m. and 11.00 a.m. on a sunny day.

Physiological and biochemical parameters of passion fruit leaves

The second internode leaf collected from healthy passion fruit plants was placed in pre-chilled extraction buffer (50 m*M* phosphate buffer with 1 % PVP, pH 7.0), homogenized by grinding on ice and centrifuged at 12,000 rpm at 4 °C for 10 min. The supernatant was used to determine the superoxide dismutase (SOD), Peroxidase (POD) and catalase (CAT) activities, malondialdehyde (MDA) content, total soluble sugar content and total soluble protein content. The measurements were done as per *Principles and Techniques of* Plant *Physiological Biochemical Experiment* (22). In brief, SOD activity was measured using nitro blue tetrazolium chloride (NBT) method and absorbance was measured at 560 nm. POD activity was determined using the guaiacol method. CAT activity was measured using the potassium permanganate method. MDA content was measured using the thiobarbituric acid (TBA) method. Total soluble sugar content was determined using the colorimetric anthrone method. Soluble protein content was measured using Folin-phenol reagent method.

The content of endogenous hormones, indole-3-acetic acid (IAA), cytokinins (ZR) and abscisic acid (ABA) were determined by using the ELISA kit from Bei Nong Wei Tian Biological Technology Co. Ltd. (Beijing, China). One g of fresh leaves was homogenised in chilled pestle and mortar using the extraction buffer, containing 80 % methanol and 1 m*M* 2,6-di-tert-butyl-4-methylphenol. The homogenate was kept at 4 °C for 4 h and centrifuged at 4,000 rpm for 5 min. The supernatant was collected into another tube and the residue was again mixed with extraction buffer and centrifuged at 4,000 rpm for 5 min. The combined supernatant was used to measure the concentrations of IAA, ZR and ABA according to the user manual procedures in the kit (13).

Data analysis

All experimental data are presented as mean ± standard error (SE). They were analysed using a one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) at a 5% level of probability. The statistical and correlation analyses were performed using the SPSS 20.0 program.

RESULTS AND DISCUSSION

Passion fruit soils toxicity to receiver plants

In soil sandwich bioassay, the passion fruit soils showed toxicity on the root growth of lettuce and passion fruit (*P. edulis*), relative to control (Fig. 2). The inhibition (%) of lettuce root length increased with the increase in the soil age, 39.84% (1 year), 54.36% (2 years) and 61.83% (3 years) and that of passion fruit, 33.17% (1 year aged), 42.13% (2 years) and 48.35% (3 years) (Fig. 2). There were significant differences ($p < 0.05$) between various plantation years. Correlation analysis showed the inhibitory effects on two receiver plants were positively correlated to the soil age (Table 2). These results showed that soil toxicity exists in passion fruit plantations.

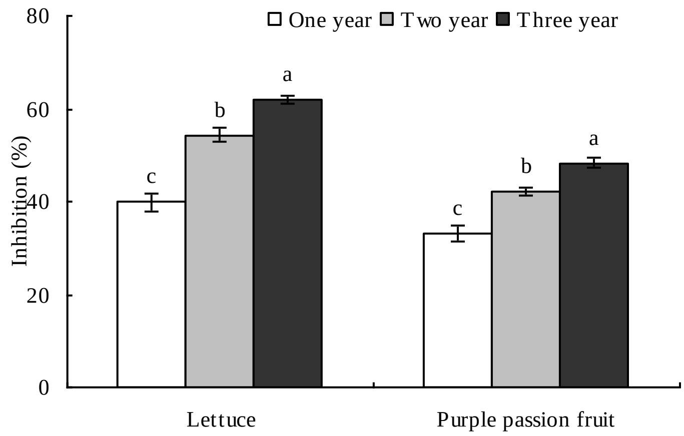

Figure 2. Inhibitory effects of test soils on the root length of lettuce and Passion fruit.
The bars represent standard errors of the mean. Different letters indicate significant differences at $p < 0.05$.

Growth of Passion fruit seedlings

The growth parameters [stem perimeter, vine length and shoot dry weight (except leaf area)] of Passion fruit seedlings, were significantly decreased, when passion fruit seedlings were replanted in the soils of older passion fruit plantations, compared with the control (Fig. 3). Correlation analysis showed the growth parameters of passion fruit were negatively correlated to the soil age (Fig. 3). Soil sickness has negative effects on crop yield and quality (3,4,6,7,8,9,11,17,18,21). Our results also indicate that the soils of older passion fruit plantations have soil toxicity, which adversely affected the growth of replanted seedlings.

Photosynthetic parameters

Photosynthetic parameters are used to evaluate the plant response to environmental stress conditions. The net photosynthetic rate, stomatal conductivity, intercellular CO_2 concentration, transpiration rate and chlorophyll content of passion fruit leaves were significantly decreased (Fig. 4) with increase in soil age, compared with control. The difference between the 2 and 3- years plantation soils was marginal. Reasons for this need further investigation. Correlation analysis showed that all photosynthetic parameters of passion fruit leaves were negatively correlated to the soil age (Table 2). These results confirmed that the soil autotoxicity had negative effects on the photosynthetic efficiency in the replanted seedlings of passion fruit.

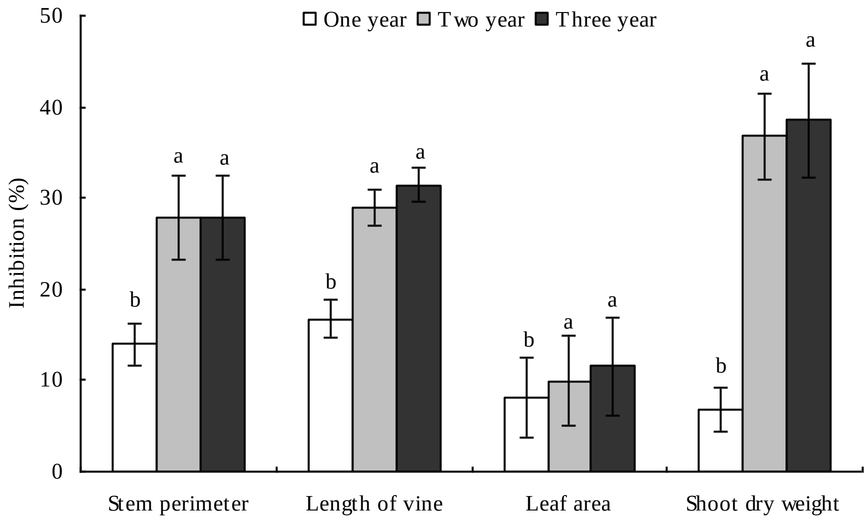

Figure 3. Effects of soils planted with passion fruit for various durations on the growth of Passion fruit seedlings
The bars represent standard errors of the mean. Different letters indicate significant differences at $p < 0.05$.

Physiological and Biochemical parameters
The production of reactive oxygen species (ROS) is often the first response of plants to abiotic or biotic stress. Plants scavenge and eliminate ROS by using antioxidant defense systems - antioxidants and antioxidant enzymes present in various subcellular compartments (5). Antioxidant enzymes (SOD, POD and CAT) eliminate the ROS and keep the oxygen free radicals of plant tissues at lower level of injury (16). As seen in Fig. 5, SOD, POD and CAT activity of passion fruit seedling leaves decreased significantly in seedlings replanted in older soils, compared with the control soil. This was accompanied by a decrease in primary metabolites, soluble carbohydrates and soluble proteins in the leaves. Lower levels of SOD, POD and CAT activity in the passion fruit leaves indicated that the older soils posed a stress condition to the replanted passion fruit plants.

MDA can damage the structure and function of organelles such as chloroplast and mitochondria and degrade DNA and other macromolecules. Hence MDA level is commonly used as a biomarker of the level of oxidative stress in an organism (16). The MDA levels in this experiment were significantly higher in the leaves of passion fruit grown in the older soils than that grown in the control soils (Fig. 5). This indicated that the older soils had a harmful affects on the replanted passion fruit plants.

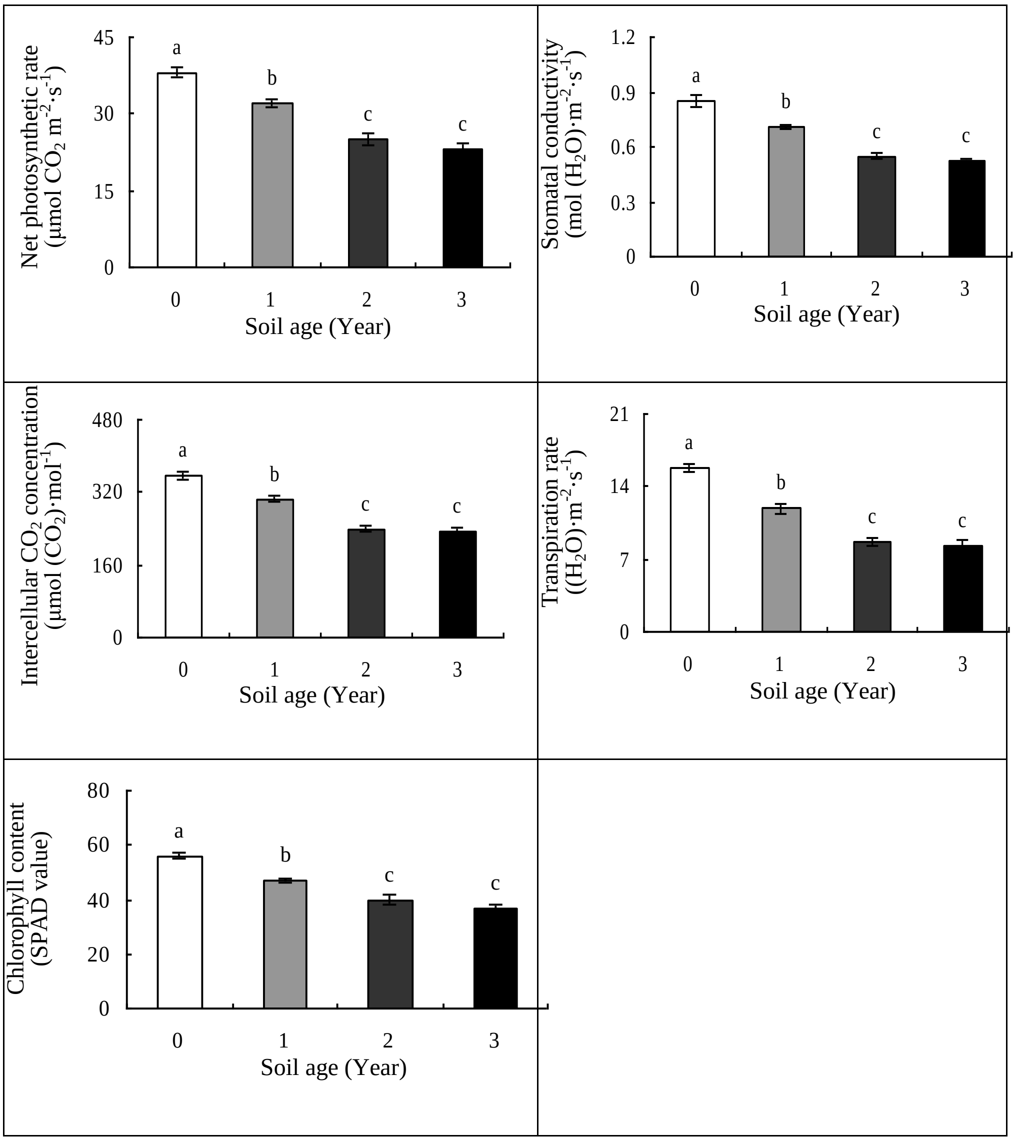

Figure 4. The photosynthesis parameters of Passion fruit seedling leaves
The bars represent standard errors of the mean. Different letters indicate significant differences at $p < 0.05$.

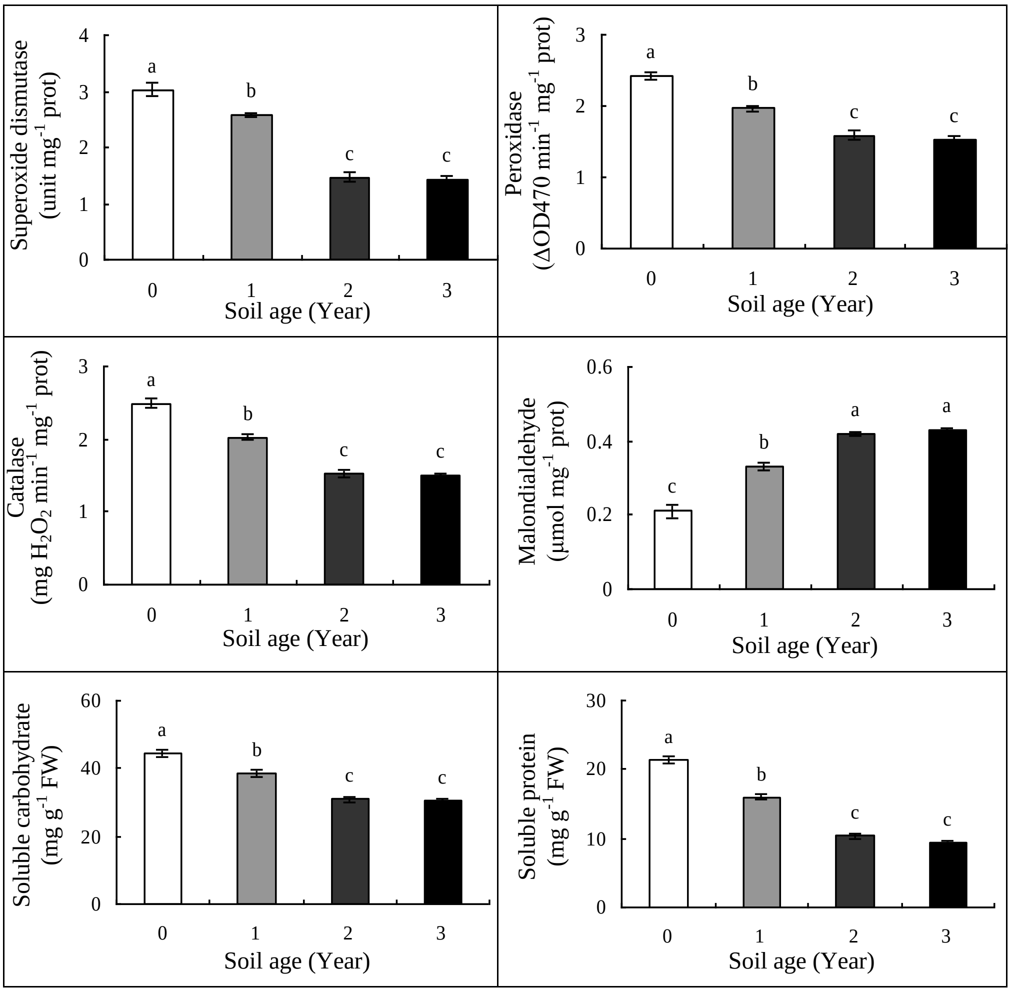

Figure 5. The Physiological and Biochemical parameters of Passion fruit seedling leaves
The bars represent standard errors of the mean. Different letters indicate significant differences at $p<0.05$. The values of soluble carbohydrate and soluble protein are on fresh weight basis and likely to slightly variable.

Plant hormones play an important role in the regulation and coordination of cell proliferation and cell elongation. IAA affects apical dominance, shoot elongation, the induction of cambial cell division and root initiation. ZR promotes cell division and determines the cell differentiation and is also associated with the senescence of plant organs, apical dominance and stomata opening. ABA is associated with desiccation tolerance, suppression of vivipary and the closure of stomata (5). As seen in Fig. 6, the levels of IAA and ZR of passion fruit leaves decreased significantly, while the level of ABA increased significantly in passion fruit leaves of seedlings replanted in the older soils, compared with the control soils.

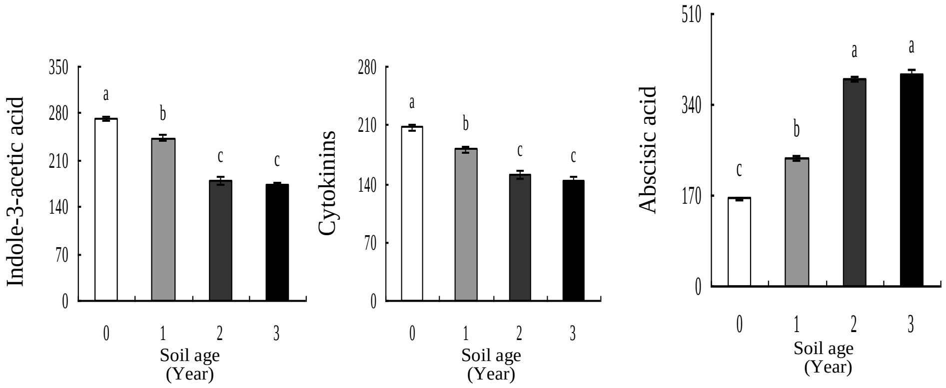

Figure 6. The levels of endogenous hormones of Passion fruit seedling leaves
The bars represent standard errors of the mean. Different letters indicate significant differences at $p < 0.05$. The values are on fresh weight basis and likely to slightly variable.

Correlation analysis showed that the activity of SOD, POD and CAT, the levels of soluble carbohydrates and soluble proteins and the levels of IAA and ZR in the passion fruit leaves were negatively correlated to the soil age and the levels of MDA and ABA were positively correlated to the soil age (Table 2).

Table 2. Correlation coefficients of the plantation age soils and the experiment parameters

	Correlation coefficient					
% Inhibition	Lettuce	Purple passion fruit				
	0.96**	0.94*				
Growth parameter	Stem perimeter	Length of vine	Leaf area	Shoot dry weight		
	-0.94*	-0.96*	-0.92*	-0.94*		
Physiological parameter	SOD	POD	CAT	MDA	Soluble carbohydrate	Soluble protein
	-0.95*	-0.96*	-0.96*	0.95*	-0.96*	-0.96**
Photosynthetic parameter	Net photosynthetic	Chlorophyll content	Stomatal conductance	Intercellular CO_2 concentration	Transpiration rate	
	-0.98**	-0.98**	-0.97**	-0.96*	-0.95*	
Endogenous hormones	IAA	ZR	ABA			
	-0.96**	-0.97**	0.96*			

Correlation coefficients indicate the plantation age soils and the experiment parameters. * and ** indicate significance level at $p < 0.05$ and $p < 0.01$, respectively.

Although soil toxicity has been widely reported in many agriculture crops, no report is available about soil toxicity and replant problem in passion fruit plantations. We have investigated herein the soil toxicity of passion fruit plantations and the effects of old soils on the growth of replanted passion fruit seedlings, as well as the affects on the photosynthetic parameters, physiological and biochemical parameters in the passion fruit leaves. All results indicated that the older soils of passion fruit plantations have negative effects on the replanted passion fruit seedlings, suggesting the presence of soil autotoxicity in passion fruit plantations. We find that the old soils have a stress factor for the replanted passion fruit seedlings, resulting in the decrease of IAA and ZR and an increase of ABA in the replanted seedlings leaves (Fig. 6). The lower levels of IAA and ZR in leaves could delay the apical dominance, shoot elongation, cell division and differentiation and root initiation. The accumulation of ABA in leaves could suppress the senescence of plant organs, shoot elongation and stimulate the closure of stomata and stress tolerance (5,16). These negative effects diminished the photosynthetic efficiency of seedlings leaves and stunted the growth of replanted seedlings (Fig. 3 and Fig. 4).

Reactive oxygen species (ROS) are often produced during the early stages of a plant resistance response (5). Under soil toxicity, the passion fruit leaves have lower contents of enzymes, which scavenge the ROS but significantly increased the levels of MDA (Fig. 5). This is apparently a reason for the poorer growth of seedlings in older soils.

Soil sickness is a complicated phenomenon related with soil matrix, root exudates, soil microbes, soil borne pathogens etc. (13) and is result of plant-soil interactions. The cause and mechanism of soil toxicity in passion fruit plantations are not fully clear. More research is necessary to solve the replant problem in Passion fruit culture.

CONCLUSIONS

In laboratory bioassay, we found that the older soils significantly inhibited the root length of lettuce and passion fruit. In the replant experiments, the older soils had negative effects on the growth of passion fruit seedlings. The photosynthetic parameters, physiological and biochemical parameters of passion fruit seedling leaves indicated that the older soils manifested stress conditions for the growth of passion fruit seedlings. These results indicate that soil toxicity exists in passion fruit plantations and soil toxicity adversely affected the growth and development of passion fruit.

ACKNOWLEDGMENT

Xiao-Ting Chen equally contributed to this research work. This study was funded by the National Key Research and Development Program of China (2016YFD0200900), the Fujian-Taiwan Joint Innovative Center for Germplasm Resources and Cultivation of Crop (FJ 2011 Program No. 2015-75), the Open Project of Key Laboratory of Agroecological Processing and Safety Monitoring (FAFU), Fujian Province (NYST-2017-01) and the Talent-Recruiting Program of Longyan University (LB2015001).

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