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Soil sickness problem in tea plantations in Anxi county, Fujian province, China

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ABSTRACT

Autotoxicity is widely reported in agro-ecosystems that declines the growth and yield but is seldom reported in old tea plantations. Rhizosphere soils of three tea cultivars of different ages were collected from a tea plantation in Anxi County, Fujian, China. In bioassay with test plants of lettuce (*Lactuca sativa* L.) and cabbage (*Brassica rapa* L.), the soils and soil extracts of > 9-years of planted age, were toxic to seed germination and seedlings growth of these test plants. In pot culture, the soils from > 9-years of planted age, also showed the effects similar to replanted tea seedlings. There was significant-positive correlation between the inhibition of test plants and the physiological parameters of tea leaves with the planted age of tea soils. These findings explain the reasons for the decline in the production and quality of tea from the old tea plantations.

Key words: Autotoxicity, bioassay, *Brassica rapa*, cabbage, *Camellia sinensis* L., *Lactuca sativa*, lettuce, physiological parameters, planted age, replant problem, rhizosphere soil, tea.

INTRODUCTION

In China, tea is produced from the Tea plant (*Camellia sinensis* L., family Theaceae) and is one of the seven necessities of Chinese life. According to tea manufacturers, there are 6-Chinese teas: white, green, red, cyan, black and yellow tea (19). The Anxi Tieguanyin tea (origin: Anxi County, Fujian Province, China) is one of the 10-Chinese tea and is most popular in south China and in southeast Asia. It is made from the Tieguanyin tea cultivar, sub-category: oolong tea and category: cyan tea. Currently Anxi county, Fujian province has following famous tea cultivars arranged in order of popularity: Tieguanyin, Huangjingui, Benshan, Maoxie, Meizhan, Dayeoolong.

The traditional tea plantations are grown for many generations and are located on terraced hillsides or mountains tops. With more income from tea, the farmers in Anxi County have been re-planting the old tea plantations. The new tea plantations are usually located on low hills or plains. New tea plants are reproduced by vegetative propagation and then transplanted at new sites. Tea plants are plucked twice, in spring and autumn. To get higher yields, chemical fertilizers and pesticides are heavily used. However, the production and quality of tea trees in reclaimed tea plantations declines after a decade due to soil sickness, which cannot be overcome by supplemental fertilizers or pesticides

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(2,13,14). To overcome this problem, old trees are removed from the old tea plantations, the land is reclaimed and then replanted after 10-years with new tea seedlings. The renewal of old tea plantations is very complex and complicated problem.

Soil autotoxicity has been reported in many agricultural and forestry systems (3,5,10,15,22). The soil sickness also occurs in fruit orchards and coffee plantations (3,5,11**)**. However, there is no report of tea replant problem and soil toxicity in old tea plantations. To understand how the soils of continuously planted tea trees affect the growth of new tea plants, we studied the toxicity of rhizosphere soils of tea trees of different ages in laboratory bioassays and the effects of old soils on the photosynthetic indices and protective enzymes (superoxide dismutase, peroxidase and catalase) of tea leaves in pot culture experiments.

MATERIALS AND METHODS

Soil sampling sites: Anxi County (longitude 117°36′−118°17′ E, latitude 24°50′−25°26′ N, elevation 650–800 m above sea level) has subtropical climate with annual mean temp: 16-21°C and annual rainfall: 1600−1800 mm. The soil samples were collected from the original plantations of Anxi Tieguanyin tea, Anxi County, Fujian Province, China (**Table 1).** The tea trees from the three areas (Tieguanyin, Huangjingui and Benshan), of different planted ages were chosen.

Table 1. The soil sampling sites, location of plantations, plant age and planted area of tea cultivars

Cultivar	Location	Plants age (Years)							
		0	1	3	6	9	15	22	25
		Area (Acres)							
Tieguanyin	Long. 117°93′E, Lat. 24°97′N	4	15	17	8	25	5	3	0.5
Huangjingui	Long. 117°99′E, Lat. 24°96′N	5	12	20	52	43	8	2	1.2
Benshan	Long. 117°94′E, Lat. 24°00′N	3	4	5	6	8	2	1.2	0.5

Note: Plant age 0 means no tea planted lands.

Rhisozphere soil samples were collected from the tea trees from each plot of one acre to collect the soils from around the tea tree roots (4). After selecting the trees, the top soil leaves were removed and soil samples were collected from the 4-5 cm radius around the tea plant and upto 10-25 cm depth. Soil samples from areas without tea trees were used as control. Three hundred g fresh soil was sampled using the method of coning and quartering to determine the soil toxicity. The rest of the soil sample was air-dried, powdered and sieved trough 60-mesh sieve. The physico-chemical characteristics of the soil samples were determined as per Bao (1). The soil is red ustoll and the physico-chemical properties are given in Table 2.

Table 2. Physico- chemical properties of tea garden soils of different ages

Parameters	Plants age (Years)							
	0	1	3	6	9	15	22	25
				Tieguanyin				
pH	5.85	5.72	5.64	5.45	5.24	4.96	4.72	4.53
Organic matter (g/kg)	9.42	10.35	10.68	11.37	12.54	11.89	13.48	13.26
Total N (g/kg)	1.62	1.54	1.36	1.48	1.23	1.48	1.26	1.37
Total P (g/kg)	1.05	0.95	1.32	1.28	1.46	1.27	1.36	1.27
Total K (g/kg)	1.76	1.49	1.38	1.27	1.69	1.94	1.65	1.48
Available N (mg/kg)	27.2	23.46	25.83	28.72	29.38	21.63	28.62	25.34
Available P (mg/kg)	79.5	84.62	73.53	69.78	76.85	91.32	71.37	78.26
Available K (mg/kg)	305.6	342.65	335.18	327.69	319.83	311.46	337.48	289.37
				Huangjingui				
pH	5.91	5.87	5.83	5.72	5.54	5.26	4.83	4.68
Organic matter (g/kg)	8.12	9.43	12.67	11.78	13.92	12.84	13.17	14.93
Total N (g/kg)	1.34	1.45	1.29	1.54	1.36	1.47	1.59	1.53
Total P (g/kg)	1.14	1.27	1.38	0.98	1.08	1.24	1.27	1.42
Total K (g/kg)	1.49	1.32	1.36	1.49	1.54	1.69	1.19	1.25
Available N (mg/kg)	23.89	21.75	24.56	26.42	27.83	23.16	22.09	23.14
Available P (mg/kg)	62.18	73.25	64.39	68.84	77.89	75.17	69.23	74.91
Available K (mg/kg)	314.17	352.83	321.36	309.16	302.27	305.52	309.37	319.58
				Benshan				
pH	5.87	5.82	5.74	5.68	5.44	5.08	4.86	4.75
Organic matter (g/kg)	6.26	9.13	12.34	13.65	11.82	13.95	12.71	12.02
Total N (g/kg)	1.45	1.38	1.26	1.17	1.42	1.25	1.31	1.19
Total P (g/kg)	1.27	1.08	1.26	1.34	1.73	1.43	1.09	1.12
Total K (g/kg)	1.92	1.73	1.84	1.62	1.74	1.79	1.85	1.62
Available N (mg/kg)	34.52	29.84	35.62	33.78	35.91	29.14	30.05	30.67
Available P (mg/kg)	85.63	79.48	81.74	84.65	88.93	79.16	80.59	82.17
Available K (mg/kg)	278.95	299.16	284.67	306.42	317.28	286.53	290.54	286.79

Soil sandwich bioassay: Soil samples of each tea tree, from 3-sites of different planted ages were collected (Table 1). The toxicity of rhizosphere soils was determined using a slightly modified version of soil sandwich bioassay method (4). In brief, 15 g fresh soil was added to 30 mL of 0.8% boiled agar solution, cooled to about 45°C, shaken well and poured into a 9 cm Petri plates. After solidification, 2 mL of 0.5% agar solution was added to cover the surface. Soil samples from the area without tea trees were used as control. Ten germinated seeds of lettuce (*Lactuca sativa*) or cabbage (*Brassica rapa*) were placed on the agar surface. There were six replicates for each test. The plates were then placed in an artificial climate box. Lettuce was cultured for 3 days at 25 ^{o}C and cabbage at 30 ^{o}C for 5 days, with 12 h (7:00 - 19:00) light of 2400 Lux light intensity. All plants were then collected separately and oven-dried at 120°C for 30 min to quickly stop enzyme activity and then at 80°C for 48 h to obtain their dry weight.

Bioassay of Soil extracts: The soils samples of each tea cultivars of different planted ages were collected as described above. Fifty g fresh soil was added to 150 mL of 80% ethanol, stirred and allowed to stand for 48 h. It was then filtered through a filter paper, concentrated to 50 mL by vacuum rotary evaporator at 50 ^oC and then diluted with double distilled water to 500 mL. Five mL of the soil extract was then added to a 250 mL beaker, lined with a filter paper and 10 germinated seeds of lettuce or cabbage were placed in each beaker. Extract from soil without tea trees was used as control. There were six replicates for each test. For culture and plant dry weight, the method used in soil sandwich bioassay was followed.

Pot Culture

The pot culture experiment was conducted in April 2013 in Anxi County tea plantation. Tea seedlings (1 year old) of 3-tea cultivars (c.v. Tieguanyin, c.v. Huangjingui and c.v. Benshan) were purchased from the Tea Institute, Fujian Academy of Agricultural Sciences, China. Rhizosphre Soil was collected from around the three tea cultivars as described above. Soil from the area without tea trees was used as control. Three kg powdered soil was added to each pot (22 cm height, 15 cm dia) and two tea seedlings were transplanted in each pot. The treatments were replicated 6 times To maintain soil moisture during the plant growth, 100 mL distilled water was added to each pot once in three days. Three g urea dissolved in 100 mL distilled water was added per pot on June 5 and November 18, 2013. One year after transplanting, the photosynthetic and physiological parameters of tea leaves were determined as under.

Measurement of photosynthetic indices of Tea seedling leaves

The second internode functional leaf of tea plants was used to measure the net photosynthetic rate and the chlorophyll content. The net photosynthetic rate (Pn) was measured using a portable photosynthesis system (LiCor-6400, LiCor Inc., Lincoln, Nebraska, USA) and a LED light source, 6400-02 at ambient CO_2 concentration under a controlled light intensity of 1000 μmoL m^{-2} s^{-1} (9). The chlorophyll content in tea leaves was determined using a SPAD-520 portable chlorophyll meter (Konica Minolta Camera Co. Ltd., Japan). The indices data were determined between 9.00 a.m. and 11.00 a.m. on sunny days and there were six replicates.

Determination of protective enzyme activity of tea leaves

The measurements were made as described in the book, *Principles and Techniques of Plant Physiological Biochemical Experiments* (18). The second functional internode leaf of tea plants was collected to measure the protective enzyme activity of tea leaves. Enzyme extract was prepared by homogenizing the 0.3 g frozen tea tree leaves from each treatment in 3 mL of phosphate buffer (PBS) (0.05 M, pH 7.0) containing 1% (w/v) polyvinyl polypyrolidone. The homogenate was filtered through a triple layer of cheesecloth and centrifuged at 15,000 x *g* at 4 ^oC for 10 min. The supernatant was used to determine the enzyme activities and protein concentration. Six replications were maintained to determine the activities of superoxide dismutase (SOD, EC 1.15.1.1), peroxidase (POD, EC 1.11.1.7) and catalase (CAT, EC 1.11.1.6). Total soluble protein content of extracts was determined using Coomassie brilliant blue method with Bovine serum albumin as standard (18).

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 percent inhibition (PI) was used to evaluate the effect of soils and soil extracts on the test plants (lattice and cabbage). It was calculated using the following formula: PI = (1 - Treatment/Control) × 100%.

RESULTS AND DISCUSSION

Rhizosphere soils

Soil sandwich bioassay showed that the inhibition in dry weight of lettuce and cabbage was < 10% by the tea tree rhizosphere soils of 1 to 9 planted years, compared to 0 year control soil (Fig. 1).

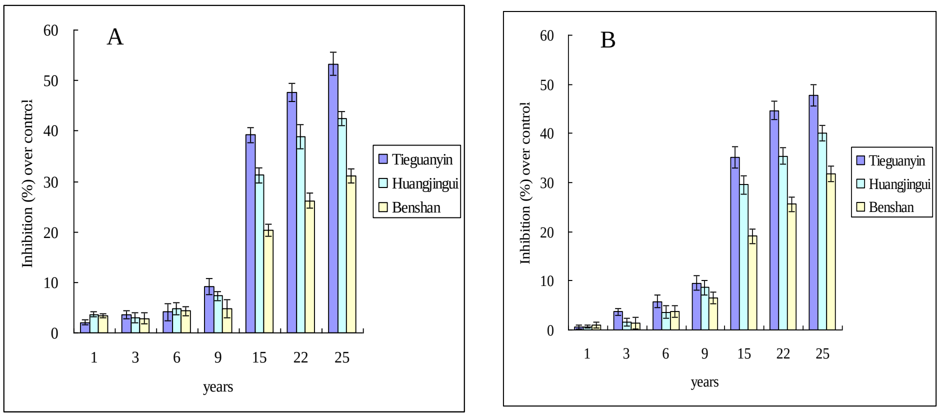
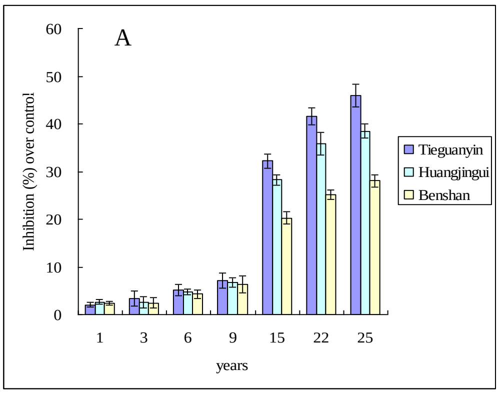


Figure 1. Inhibitory effects of rhizosphere soils from tea trees of various ages from 3-sites on dry weight of lettuce and cabbage.
The bars represent standard errors of the mean (n=6). A: Lettuce, B: Cabbage

However, the inhibition (%) increased sharply with the increase in soil planted ages from 15 to 25 years. The rhizosphere soils of Tea trees c.v. Tieguanyin with 15, 22, and 25 years planted age reduced the lettuce dry weight by 39.20%, 47.65%, and 53.29%, respectively and that of cabbage by 35.19%, 44.68%, and 47.75%. The rhizosphere soils of tea trees c.v. Huangjingui of 15, 22, and 25 years planted age, reduced the lettuce dry weight by 31.23%, 38.86% and 42.45%, respectively, and cabbage dry weight by 29.52%, 35.42%, and 40.06%. The rhizosphere soils of tea trees c.v. Benshan with 15, 22, and 25 years planted age reduced the dry weight of lettuce by 20.27%, 26.12%, and 31.07% respectively and that of cabbage by 19.06%, 25.57%, and 31.76%. These results suggest that the soil toxicity of all 3-tea cultivars increased with age and become more harmful after 9-planted years of age and afterwards increased with increasing years of planted age.

Rhizosphere soil extracts

In laboratory bioassay, the extracts of tea tree rhizosphere soils were less inhibitory to dry weight of lettuce and cabbage from the tea trees of 9-years than 0 year control soil. However, the extracts from the rhizosphere soils of the three tea cultivars with 15, 22, and 25 planted years, increased with the age (Fig. 2). The inhibition (%) in dry weight of lettuce and cabbage increased by 20% - 46% and by 20% - 43%, respectively.



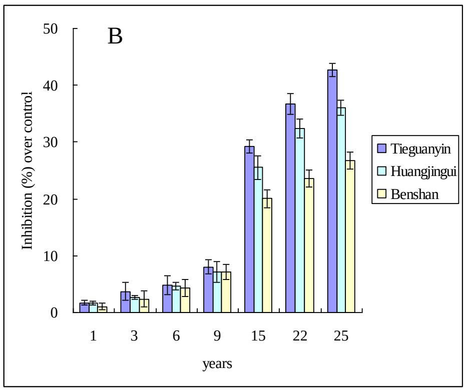


Figure 2. Inhibitory effect of rhizosphere soil extracts from tea trees of various ages from 3-sites on dry weight of lettuce and cabbage.
The bars represent standard errors of the mean (n=6). A: Lettuce, B: Cabbage

The effects of the soil extracts on receiver plants were similar to the effect of soils. These results showed that the chemical substance in the rhizosphere soils of tea trees was responsible for the soil toxicity. Suzuki and Waller (12) reported that caffeine and theobromine released into the soil by the tea roots inhibited the shoot and root of tea seedlings. Cao *et al*. (2) reported that accumulation of phenolic acids in old tea plantations resulted in tea autotoxicity. Some phenolic compounds have also been reported as autotoxins in apple, citrus, coffee and tea plantations (5). In tea tree management, normally the trees are prunned after each plucking and the plant residues are left around the tree base as organic fertilizer. This practice perhaps results in the accumulation of some autotoxins from the plant residues in to the rhizosphere soils of tea trees.

Physiological parameters

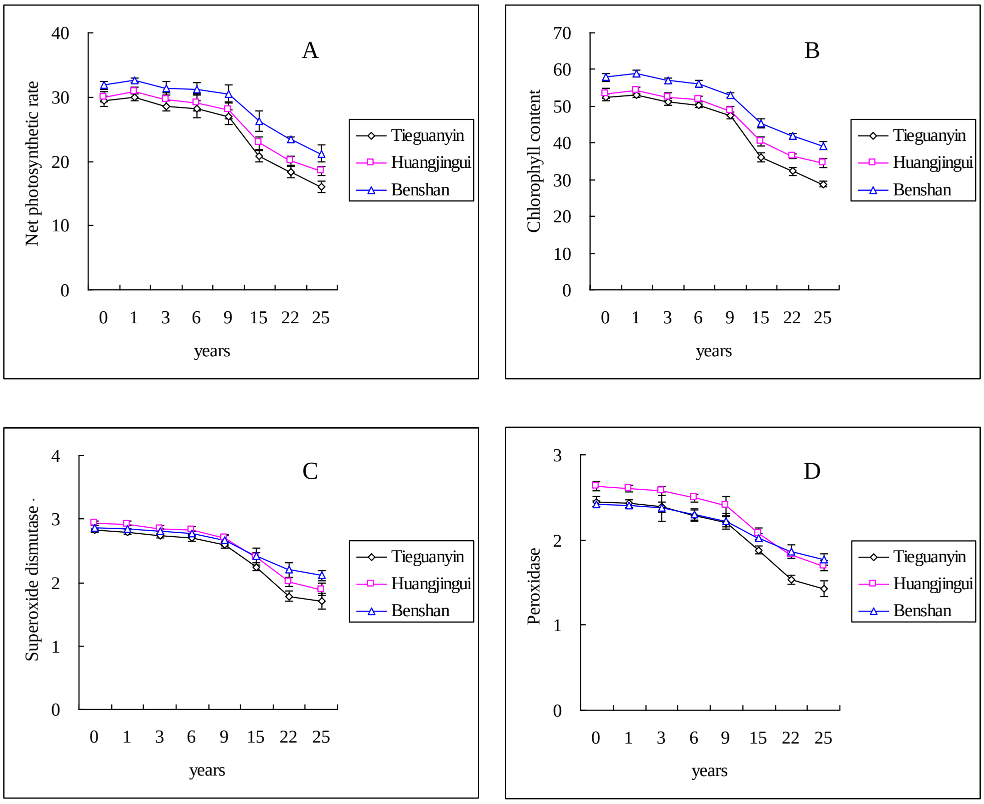
The physiological parameters of tea leaves, [net photosynthetic rate, chlorophyll content, and activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT)], decreased with the increase in the age of tea trees. It is apparent that there is a turning point in each parameter at 9-planted years (Fig. 3). Correlation analysis showed that the planted age of the 3-tea cultivars rhizosphere soils, was significantly and positively correlated with the inhibition (%) by soils and soil extracts on lettuce and cabbage, net photosynthetic rate, chlorophyll content and enzyme activities in tea leaves (Table 3). It appears therefore, that the primary and secondary metabolism in tea plants is greatly influenced by the photosynthesis, chlorophyll and enzyme activities, which subsequently affects the level of tea polyphenols, total catechins, soluble sugar, caffeine and amino acids in tea leaves (7). Our results suggest that the negative influence of autotoxicity in old tea plantations on photosynthesis, chlorophyll and enzyme activities in tea leaves not only decreases the tea production but also lowers the tea quality.

Table 3. Correlation analysis of the planted age of Tea tree rhizosphere soils with the inhibition (%) of soils and soil extracts on lettuce and cabbage and physiological parameters of tea leaves

Cultivar	PI of soil		PI of soil extract		Pn	Chl	SOD	POD	CAT
	lettuce	cabbage	lettuce	cabbage					
Tieguanyin	0.97**	0.98**	0.97**	0.99**	0.98**	0.99**	0.99**	0.98**	0.98**
Huangjingui	0.97**	0.98**	0.97**	0.99**	0.99**	0.99**	1.00**	0.99**	0.98**
Benshan	0.97**	0.99**	0.98**	0.99**	0.99**	0.99**	1.00**	0.99**	0.98**

** Correlation is significant at 0.01 level. PI : Percent inhibition; Pn : net photosynthetic rate; Chl : chlorophyll content, SOD : superoxide dismutase, POD : peroxidase, CAT : catalase.

Autotoxicity has been widely reported in agro-ecosystems, which leads to a decline in growth and yield, especially in monoculture crops (3,5,11,20,21,23,24). In annual crops, autotoxicity can be partially overcome by a proper fallow period or by a proper rotation and intercropping. This is, however, not possible in tea plantations because the tea tree is a perennial plant. In Anxi County, the new tea plantations are in pure stand with high-density and high output level (13,15). In such situations, autotoxic chemicals from the root exudates and chemicals from plant residues accumulate in the rhizosphere soils of tea trees, after long-term cultivation leading to autotoxicity. Direct evidence to this is however lacking. The accumulated autotoxins in the rhisosphere soil can decrease the CO_2 assimilation and PSII electron transport efficiency (5). Phenolic compounds inhibits the photosynthesis and antioxidant enzymes, cell division and DNA synthesis in the root apical meristem, disturbs the ion uptake and cell membrane functions in the roots in the short duration cucumber (21,23,24). Root exudates and plant residues in continuous crop monoculture reduces the microbial competition in the root zone by decreasing the biodiversity in fungi and bacteria and increasing the soil-borne pathogens, thus increasing the soil sickness (5,8,15,20,26). The decomposition of residues from prunned tea trees commonly used as an organic fertilizer amendment in tea management could also increase the soil pH not only in surface soil layers but also in subsoil layers, thus increasing the aluminumions in soil, which significantly decreases the amino acids and polyphenol contents in tea leaves (16). Results of our previous study (15) showed that the microbial biomass and respiration, the bacterial number in the rhizospheric soils of c.v. Huangjingui decreased significantly as the tea tree age increased, which was positively-correlated with the soil pH. The number of fungi increased significantly, which was negatively-correlated with the soil pH (15). Soil sickness in agroecosystems is complex problem involving various factors viz., soil physico-chemical properties, soil nutrients unbalance, autotoxins



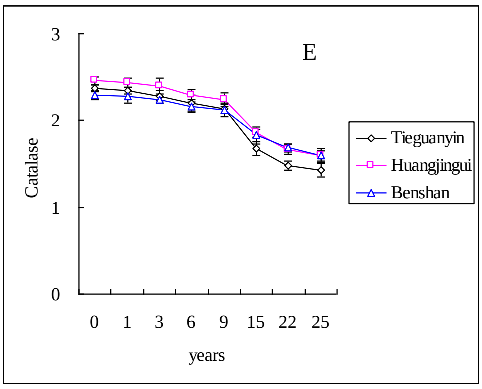


Figure 3. The physiological parameters of tea seedlings cultured in the tea tree soils with different planted ages.

The bars represent standard errors of the mean (n=6).
A : Net photosynthetic rate (CO_2·μmol·m^{-2}·s^{-1}) B : Chlorophyll (SPAD value); C : superoxide dismutase (unit·mg^{-1} protein); D : peroxidase (△OD470 min^{-1}·mg^{-1} protein); E : catalase (mg (H_2O_2)·min^{-1}·mg^{-1} protein).

production and accumulation, as well as change in microbial diversity in the rhizosphere soil (3,5,6,7,9,11,15,16,17,25). Therefore, it is necessary to study the soil sickness in tea plantations and determine the mechanism of its action by multidisciplinary approach.

CONCLUSIONS

We found that strong toxicity exists in tea tree rhizosphere soils of > 9 years age. The chemicals in the soil released by the tea plant as well as the plant residues, were responsible for the soil toxicity and poor performance of tea plants. Pot culture experiments are in agreement with this conclusions. These findings could help the tea farmers to know the reasons for the decrease in yield and in quality in old tea plantations and to choose suitable methods to overcome the soil sickness problem.

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