https://doi.org/10.26651/2017-42-1-1105 0971-4693/94 Euro 20.00
Allelopathy Journal 42 (1): 49-64 (September, 2017) *International Allelopathy Foundation* 2017
Table: 5, Figs : 9

Allelopathic effects of persimmon (*Diospyros kaki*) leaves extracts on germination, seedling growth and enzymatic activities of receptor plants

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(Received in revised form: July 17, 2017)

ABSTRACT

We studied the mechanism of allelopathic effects of fresh persimmon (*Diospyros kaki*) leaves extract on: (i). seed germination, seedling growth and chlorophyll content of seedlings of receptor plant and (ii). analysed the activity of superoxidedismutase (SOD), peroxidase (POD), catalase (CAT) and root activity of receptor plants and content of malondialdehyde (MDA). The aqueous extracts of persimmon leaves had variable allelopathic effects on the receptor plants. All aqueous extracts significantly inhibited the lettuce and cabbage than control, but only high concentrations (50 mg and 100 mg) were inhibitory to mung bean, millet, rapeseed and corn. The extracts at 100 mg concentration were most inhibitory to lettuce and cabbage than other receptor plants. The growth and development of plants were significantly affected and the roots activity decreased with the increasing extracts concentration. The effects of extracts from persimmon leaves on activities of CAT, SOD, POD and the content of MDA of receptor plants were determined during the lettuce and cabbage seedling growth. The extracts had significant effects on the activities of CAT, SOD and POD in receptor plants, which *in vivo* first increased and then decreased. Of all the enzymes assayed (SOD, POD, CAT), the SOD and the POD activity cooperated to remove the reactive oxygen radicals. The allelopathy affected the activity of protective-enzymes of receptor plants and broke the structure and function of membrane. So the balance of activate oxygen metabolism was broken, the cell membrane was destroyed and the content of MDA increased.

Key words: Allelopathic mechanism, allelopathy, bioassay, cabbage, *Diospyros kaki*, enzymes activities, leaf extract, millet, mungbean, persimmon, rapeseed, root activity.

INTRODUCTION

Allelopathy plays significant role in natural and managed ecosystems (8,12), especially in agroecosystems and affects the growth, quality and quantity of crops (16,23). Plants have evolved several strategies to interact with other organisms during self defence, sexual attraction, symbiosis and other developmental processes (22). Allelopathic plant interactions have either harmful or beneficial effects and are evaluated by testing some physiological mechanisms that inhibited/ stimulated the seed germination, plant growth and development due to the presence of another plant (24,27). Thousands of plant-derived allelochemicals have already been identified, which are active against weeds (10), fungal pathogens (18), nematodes (2) and insects (13). An equal or potentially larger number of

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allelochemical compounds have been isolated from soil microbes (12). Allelochemicals are low molecular weight compounds excreted from plants during the yield of other crops or the same crop (1). In past 20 years, allelopathy has gradually become an important research area worldwide. It mainly includes the separation and indentification of allelopathy material, allelopathy biological detection method, allelopathic germplasm resources evaluation and allelopathy genetic characteristics research.

Photograph Persimmon (*Diospyros kaki* L.f)

Persimmon (*Diospyros kaki* L.f) origin is in China (Chang Jiang River basin). It is being cultivated since long time, as its fruit, leaf flower, peel root and pedicel are used in medicine. Allelopathy or autotoxicity has been assessed using the bioassays of plant or soil extracts based on seed germination and seedling growth. This study aimed to test *in-vitro* allelopathic effects of leaf extracts of persimmon trees on receptor plants metabolism, elucidate the physiological effects of allelochemicals and to obtain evidence of allelopathy in persimmon, thereby providing a theoretical basis for its planting between the fruit trees rows in orchards.

MATERIALS AND METHODS

Persimmon leaves extract

Fresh leaves of 6-years old persimmon tree were collected in May 2016, from the Nursery, Weifang Engineering Vocational College, Qingzhou, Shandong, China (Latitude 36º56', Longitude 118º46', mean height above sea level 485.25m, mean maximum temp. 26.3℃, mean minimum temp. -2.6℃ and annual rain fall 664mm). They were washed with distilled water, cut into 2-3 cm pieces and dried in shade for 96 h at room temp. (25 ^{o}C). The leaves were then powdered using a mortar and pestle. One hundred g dried leaf powder was mixed with 1.0 L distilled water in conical flasks and kept on shaker (100 rpm) for 48 h at room temperature (25 ^{o}C). During this process, the conical flasks were kept free of contamination, to reduce microbial infection. The mixture was then filtered first through

one-layer of filter paper and then through three-layers of filter paper and then centrifuged at 3000 rpm and again filtered through filter paper. Then it was diluted with distilled water to give 12.5, 25, 50, 100 mg/ml concentrations and stored at 4 ^{o}C. The 5 ml persimmon leaf extracts of 100 mg/ml concentration was membrane-filtered (0.45 µm) for the GC-MS analysis.

Bioassay

The seeds of all test crops viz., [lettuce (*Lactuca sativaL. var. asparaginap Bailey*), mung bean (*Vigna radiata* (L.) Wilczek), millet (*Setaria italica (L.) Beauv. Var. Germanica* (Mill.) Schrad), cabbage (*Brassica pekinensis L.*), rapeseed (*Brassica campestris* L.) and corn (*Zea mays L. sp.*)] were purchased from College of Horticulture, Xi'an, Shaanxi, China. Before bioassays, the plant seeds were surface-sterilized by immersing for 10 min in 95% (v/v) ethanol followed by 30 min in 0.3 % hydrogen peroxide. Seeds with cracks in seed coat and internal microbial contamination absorb water more quickly than intact seeds float on the water surface, where they were collected and discarded. The remaining seeds were rinsed 5 times with sterile, double-distilled water (ddH_2O). Petri dish (11 cms dia) two-layers of filter paper method were used for seed germination bioassay experiments. In germination tests, 50 sterilized seeds of each receptor plant were evenly spaced on the filter paper and covered by two-layers of filter paper in petri dishes. Then 6 ml extract of various concentrations (12.5, 25, 50, 100 mg/ml) as per treatments were added per petri dish and distilled water was used as control. Then all petri plates were kept in incubator in dark at 25 ± 1℃. Treatments were replicated thrice in completely randomized design. Germination was recorded every 24 h, indicated by emergence of the 1-2 cm radicle from the seed coat. To maintain moisture in the petri dishes, 3 ml water was added daily for 10d, thereafter, seedlings growth and seedling chlorophyll content were measured from 10-randomly selected seedlings.

Chlorophyll content

We took 0.1 g fresh leaves from each culture dish into mortar, plus a small amount of quartz sand and 0.5 ml acetone (95%) ground into homogenate. Then added 5 ml acetone (80%) and filtered into 25 ml volumetric flask and made the volume with 95% acetone. Acetone (95%) was used as the blank sample, the absorbance was determined at wavelength of 663 nm and 645 nm and chlorophyll content was calculated (17).

SOD activity: Superoxide dismutase (SOD) activity was measured using the nitroblue tetrazolium (NBT) reduction method of Zou (31). Five g turnip leaves, treated with walnut root exudates were grounded into powder in the pre-cooled mortar, followed by 2 mL phosphate buffer (50 mmol·L^{-1}, at pH 7.8). When the sample was sufficiently homogenized, 4 ml phosphate buffer was added to wash the mortar, then, additional 10 mL phosphate buffer was added. The solution was transferred into a plastic centrifuge tube and centrifuged at 10000r·min^{-1} for 20 min at 4 ºC by refrigerated centrifuge (SIGMA). The SOD reaction system consisted of following components: 1.5 mL phosphate buffer (50 mmol·L^{-1}, at pH 7.8), 0.3 mL methionine (Met) (130 mmol·L^{-1}), 0.3 NBT (750 µmol·L^{-1}), 0.3 mL EDTA-Na2 (100 µmol·L^{-1}), 0.3 mL riboflavin (20 µmol·L^{-1}), 0.1 mL enzyme solution and 0.5 mL distilled water. The SOD activity was determined by U-2001 spectrophotometer (HITACHI) by using colorimetric determination at 560 nm. The

activity of SOD was given as $U \cdot g^{-1}FW \cdot min^{-1}$. After centrifugation, the supernatant was used in separate assays of POD activity and CAT activity.

POD activity: The peroxidase (POD) activity was assayed as per method of Zou (31). The POD reaction system consisted of following components: 0.9 mL guaiacol (20 $mmol \cdot L^{-1}$), 1 mL phosphate buffer (50 $mmol \cdot L^{-1}$, pH 7), 0.1 mL enzyme solution and 1 mL H_2O_2 (30 $mmol \cdot L^{-1}$). The POD activity was determined by U-2001 spectrophotometer at 470 nm. The activity of POD was calculated by recording the change in OD 470 values at 3 min intervals after the initial biochemical reaction. POD activity was shown as $U \cdot g^{-1}FW \cdot min^{-1}$.

CAT activity: The catalase (CAT) activity was determined as per method of Zou (31). The reaction system for CAT included the following components: 1.9 mL phosphate buffer (pH 7, 50 $mmol \cdot L^{-1}$), 0.1 mL reserved supernatant (the same as for SOD activity assay) and 1 mL distilled water. The colorimetric determination of CAT was conducted by U-2001 spectrophotometer (HITACHI) at 240 nm. The biochemical reaction was started by adding 0.3 mL H_2O_2 (30 $mmol \cdot L^{-1}$) in the reaction system. The CAT activity was calculated by changes in OD 240 values at 3 min intervals after the initial biochemical reaction and the units shown are $U \cdot g^{-1}FW \cdot min^{-1}$.

MDA activity: Level of lipid peroxidation was expressed as the content of MDA as per Zhang *et al*. (29). Three g leaves were grounded into powder in the pre-cooled mortar, followed by 2 mL phosphate buffer (50 $mmol \cdot L^{-1}$, at pH 7.8). When the sample was sufficiently homogenized, 3 mL phosphate buffer was added to wash the mortar, then added 5 mL Thiobarbituric acid (TBA) (50 $mmol \cdot L^{-1}$) and thereafter, all solution was boiled in water bath for 10 min, then transferred into a plastic centrifuge tube, centrifuged at 3000 $r \cdot min^{-1}$ for 20 min. The supernatant after centrifugation was used to measure the MDA activity assay. The MDA activity was determined by U-2001 spectrophotometer (HITACHI) by using Colorimetric Determination with U-2001 spectrophotometer (HITACHI) at 600 nm, 532 nm and 450 nm colorimetric wavelength. The activity unit of MDA is given as $mmol \cdot g\ FW^{-1}$.

Determination of root activity: The activity of root system was determined as per chloride three phenyl four nitrogen (TTC) method (32). Took 0.5 g root sample, put it in a small beaker, add 0.4% TTC solution and phosphoric acid buffer (pH 7.0) each 5 mL, so that the root is fully immersed in the solution, dark insulation for 3 h at 37^{o}C and then immediately add 1 mol/L sulfuric acid 2 mL, to terminate the reaction. At the same time make a blank test, firstly add sulfuric acid, and then root samples, 37 ^{o}C after incubation with dark sulfuric acid, the concentration and the operation steps as above). Remove the root, dry paper moisture with filter, place in mortar, add ethyl acetate 3-4mL, do full grinding to extract three benzoyl. Then put the red extraction into a graduated tube, with a small amount of ethyl acetate washing the residue 2-3 times, both in scale test tube. In last, add ethyl acetate to make total l0 mL, use spectrophotometer at wavelength of 485 nm colorimetric with blank test as the reference, absorbance was measured, check the standard curve, obtain the amount of TTC reduction.

TTC reduction strength= Amount of TTC reduction (g)/[root weight (g)×time (h)]

Data analysis

The statistical analysis of all data was done using one-way analysis of variance (ANOVA) test in SPSS 16.0. Differences among means were detected with Duncan's multiple range test at 5% level.

RESULTS AND DISCUSSION

Seed germination

The persimmon leaf extracts inhibited the seed germination of test plants and the inhibitory effects increased with the extract concentration (Table 1). At all concentrations, the persimmon leaf extracts inhibited the seed germination in all 6-types of test plants. The leaf extracts at 50 mg/ml and 100 mg/ml concentrations significantly inhibited the seed germination in all six receptor plants compared with control. At low concentration (12.5 mg/ml), the seed germination of all receptor plants differed significantly than control, except for cabbage.

Table 1. Effects of persimmon leaves extracts on seed germination of six receptor plants at 10-days after sowing.

Receptor plants	Seed germination (%)				
	100mg	50mg	25mg	12.5mg	Control
Lettuce	7.53±0.04a	8.91±0.06a	28.52±0.03ab	36.56±0.43ab	42.55±0.02a
Mung Bean	43.5±0.56a	51.9±0.04ab	54.18±0.07ab	61.71±0.06a	87.5±0.05a
Millet	19.32±0.31b	47.41±0.12a	64.29±0.08a	79.33±0.02b	89.94±0.31ab
Cabbage	18.95±0.15ab	32.64±0.33ab	43.63±0.16a	46.27±0.11a	48.52±0.21a
Rapeseed	2.87±0.06ab	3.93±0.41a	7.82±0.42b	8.41±0.17ab	12.77±0.04b
Corn	14.26±0.44b	59.16±0.09a	74.31±0.16a	78.93±0.46b	84.65±0.76a

Each value is mean of three replicates. Means within each column followed by the same letter are not significantly different at 5% level as determined by Duncan's multiple range test.

The allelopathic activity depended on the persimmon leaf extracts concentrations (Fig. 1), which may be attributed to the different allelopathic effects of various allelochemicals present in persimmon leaves. Seed germination in corn was most inhibited (83.15%) at leaf extracts of 100 mg/ml concentration. The inhibition in lettuce was 79.06% with leaf extracts 50 mg/ml concentration. While there was 38.76% and 34.14% inhibition in rapeseed with leaf extracts 25 mg/ml and 12.5 mg/ml concentrations, respectively. The minimum inhibition in seed germination in 6-receptor plants was 50.28% in mung bean with leaf extracts 100 mg/ml concentration over the control, 30.11% in corn with leaf extracts of 50 mg/ml concentration and 10.07% and 4.63% in cabbage with leaf extracts 25 mg/ml and 12.5 mg/ml concentration, respectively.

Seedling growth

The persimmon leaf extracts at 50 and 100 mg/ml concentrations significantly inhibited the seedling growth of all six receptor plants (Table 2, Fig. 2). The leaf extracts of 100 mg concentration drastically inhibited (79.25%) the seedling height in millet. The inhibition in mung bean was 52.05% at 50 mg/ml concentration. While there was 21.64% and 18.44% inhibition in cabbage and with leaf extracts at 25 mg/ml and at 12.5 mg/ml concentrations, respectively. The minimum inhibitory effects on seedling height in six test plants were 51.89%, 0.63% and 0.12% in corn with leaf extracts at 100 mg/ml, 25 mg/ml,

and 12.5 mg/ml concentrations, respectively, compared with control and 22.54% in rapeseed with the leaf extracts of 50 mg/ml concentration.

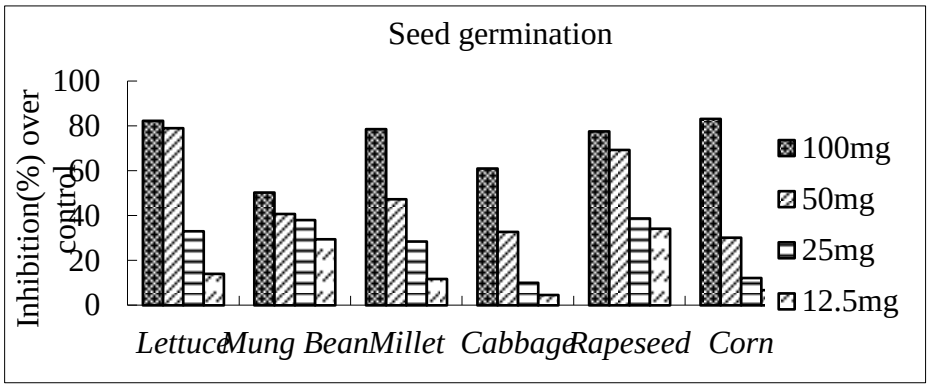

Figure 1. Effects of persimmon leaves leachate on germination of test plants.

Table 2. Effects of persimmon leaves extracts on the seed growth of six receptor plants at 10-days after sowing.

Receptor plants	Seed growth (cm)				
	100mg	50mg	25mg	12.5mg	Control
Lettuce	2.08±0.26a	3.71±0.37b	4.98±0.31b	5.05±0.03a	5.87±0.02a
Mung Bean	2.99±0.16ab	5.72±0.23a	10.91±0.42b	11.69±0.21ab	11.93±0.17a
Millet	1.84±0.32b	4.34±0.28b	7.78±0.26ab	8.16±0.18b	8.87±0.01ab
Cabbage	1.17±0.10ab	2.13±0.15b	2.93±0.21b	3.05±0.26b	3.74±0.18b
Rapeseed	1.23±0.18a	3.16±0.11a	3.72±0.31a	3.99±0.09ab	4.08±0.05a
Corn	3.81±0.14a	5.64±0.06ab	7.87±0.27ab	7.91±0.05a	7.92±0.07a

Each value is mean of three replicates. Means within each column followed by the same letter are not significantly different at 5% level as determined by Duncan's multiple range test.

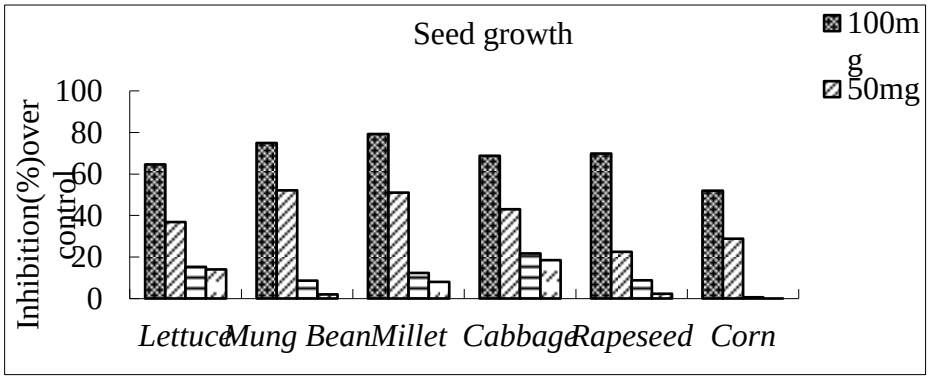

Figure 2. Effects of persimmon leaves leachate on the seed growth of test plants.

The root length was inhibited most (91.91%) in mung bean (Table 3) with leaf extracts of 100 mg/ml concentration over control. The inhibition in cabbage was 66.28%

and 25.22% with leaf extracts of 50 mg/ml and 25 mg/ml concentrations, respectively and 14.93% in lettuce with leaf extracts of 12.5 mg/ml concentration than control (Fig. 3). The minimum inhibitory effects on root length in the six plants were 76.57% in lettuce with the leaf extracts of 100 mg/ml concentration than control, 22.02% in rapeseed with leaf extracts of 50 mg/ml concentration and 1.41% and 0.83% in mung bean with the leaf extracts of 25 mg/ml and 12.5 mg/ml concentrations, respectively.

Table 3. Effects of persimmon leaves extracts on the root growth of six receptor plants at 10-days after sowing.

Receptor plants	Root growth (cm)				
	100mg	50mg	25mg	12.5mg	Control
Lettuce	1.93±0.23ab	3.97±0.32a	6.29±0.82a	7.01±0.07a	8.24±0.01a
Mung Bean	0.98±0.12b	6.82±0.08ab	11.94±0.17a	12.01±0.11ab	12.11±0.18b
Millet	0.67±0.43a	2.85±0.71a	6.54±0.04a	7.12±0.25b	8.03±0.05ab
Cabbage	0.63±0.18ab	2.34±0.10ab	5.19±0.21a	5.98±0.38a	6.94±0.12a
Rapeseed	1.95±0.07b	7.01±0.63a	8.12±0.07b	8.29±0.31a	8.99±0.46ab
Corn	1.99±0.14a	8.15±0.45a	11.83±0.54ab	11.94±0.09ab	12.12±0.88ab

Each value is mean of three replicates. Means within each column followed by the same letter are not significantly different at 5% level as determined by Duncan's multiple range test.

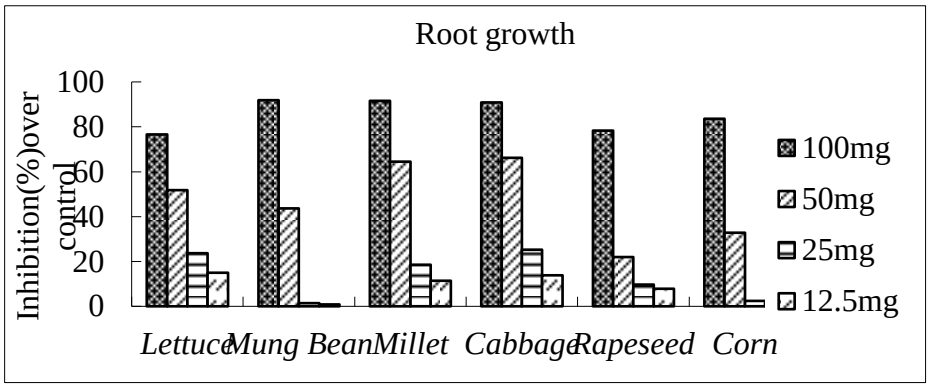

Figure 3. Effects of persimmon leaves leachate on the root growth of test plants.

Seedlings chlorophyll content

The persimmon leaf extracts decreased the chlorophyll content significantly in seedlings of millet by 89.19% (Table **4**) and 76.57% at 100 mg/ml and 50 mg/ml concentrations, respectively, over the control. While the decrease in lettuce was 44.48% and 34.73% at 25 mg/ml and 12.5 mg/ml concentrations, respectively (Fig. **4**). The minimum inhibitory effects on the seedling chlorophyll content in six receptor plants were 73.89% in lettuce with the leaf extracts at a concentration of 100 mg/ml, 46.18% in mung bean with the persemmon leaf extracts at 50 mg/ml, 15.79% concentration in rapeseed 25 mg/ml concentration and 2.71% in corn at 12.5 mg/ml concentration compared with control.

The different concentrations of leaf extracts affected the receptor plants to varying degrees, with concentration-dependent effects. All extracts concentrations (100 mg/ml, 50

mg/ml, 25 mg/ml, and 12.5 mg/ml) significantly reduced the seed germination, seedling growth and the seedlings chlorophyll contents in lettuce and cabbage than control, but only high concentrations (100 mg/ml and 50 mg/ml) reduced the seedling growth and the chlorophyll content in mung bean, millet, rapeseed and corn. The inhibitory effects of leaf extracts were stronger in lettuce and cabbage than other receptor plants, hence, lettuce and cabbage are ideal receptor plants for in-depth researches.

Table 4. Effects of persimmon leaves extracts on seedling chlorophyll content of six receptor plants at 10-days after sowing.

Receptor plants	Seedling chlorophyll content(mg/L)				
	100mg	50mg	25mg	12.5mg	Control
Lettuce	2.06±0.21a	3.34±0.08a	4.38±0.09ab	5.15±0.23a	7.89±0.55a
Mung Bean	1.92±0.09b	6.27±0.62a	7.46±0.51ab	10.18±0.94b	11.65±0.19b
Millet	1.24±0.17b	2.69±0.77b	8.41±0.38a	10.06±0.12ab	11.48±0.28ab
Cabbage	0.97±0.01b	1.96±0.65ab	4.92±0.19a	5.78±0.01ab	6.49±0.22ab
Rapeseed	4.82±0.34a	13.63±0.49ab	22.34±0.67ab	25.81±0.31a	26.53±0.06b
Corn	4.53±0.15ab	19.66±0.11b	30.27±0.31a	32.85±0.16ab	37.84±0.43a

Each value is mean of three replicates. Means within each column followed by the same letter are not significantly different at 5% level as determined by Duncan's multiple range test.

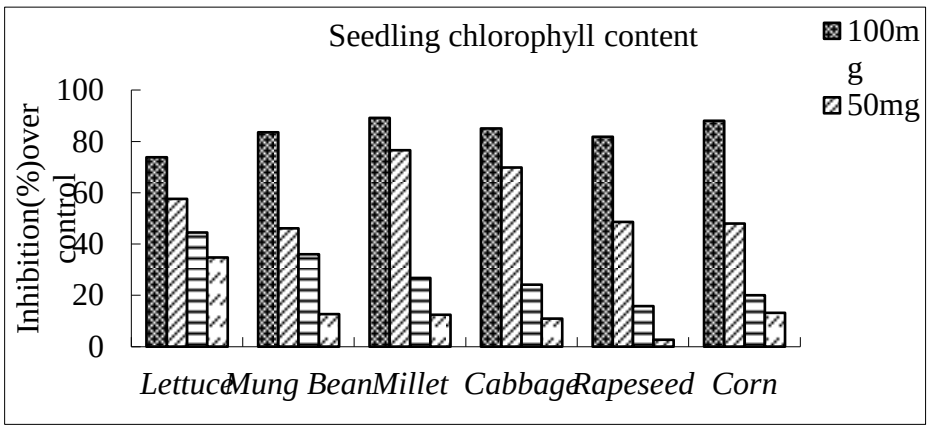

Figure 4. Effects of persimmon leaves leachate on seedling chlorophyll content of test plants.

SOD activity: SOD is one of the key enzymes. In lettuce, the activity of SOD was higher than control at 12.5 mg/ml and 25 mg/ml concentration (Table **5**). The activity in cabbage was higher than control, when the concentration was < 12.5 mg/ml. When concentration was > 12.5 mg/ml, the activity of SOD in cabbage started to decrease. The activity in cabbage at 25 mg/ml was lower than control (Fig. **5**). However at 100 mg/ml concentration, the activity was decreased in lettuce (22.29%) and cabbage (56.57%) than control ($P<0.05$). Thus at low concentration, the receptor plants could mobilize the SOD to resist the external stress. But the ability of SOD to mobilize itself is limited, when the concentration increases to a certain value, the degree of external stress would be more powerful than the ability of SOD to mobilize itself, then the activity could be weakened by external stress.

Table 5. Effect of persimmon leaves extracts on antioxidant enzyme activity of lettuce and cabbage.

Receptor plants	Control	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml
	SOD activity/U.g^{-1}FW.min^{-1}				
Lettuce	11.262±0.05a	11.517±0.09a	14.903±0.06b	9.821±0.03a	8.753±0.04a
Cabbage	26.298±0.02b	27.483±0.01ab	22.311±0.02a	15.792±0.01b	11.419±0.07b
	POD activity /U.g^{-1}FW.min^{-1}				
Lettuce	37.278±0.01a	35.309±0.03ab	32.817±0.02a	23.120±0.01b	18.429±0.03a
Cabbage	61.146±0.03a	68.573±0.08b	55.212±0.01ab	45.465±0.09ab	33.781±0.04a
	CAT activity /U.g^{-1}FW.min^{-1}				
Lettuce	33.69±0.02a	36.54±0.03ab	34.07±0.05a	19.11±0.03a	17.96±0.07ab
Cabbage	31.27±0.06b	54.13±0.05ab	39.41±0.04ab	31.18±0.09a	23.01±0.03ab
	MDA content/mmol·g FW^{-1}				
Lettuce	1.93±0.07a	2.23±0.01a	2.84±0.01a	3.06±0.03ab	5.35±0.03a
Cabbage	2.75±0.02ab	3.81±0.03b	4.37±0.06b	4.79±0.08ab	6.68±0.01ab
	Root activity/mgTTC/g.h				
Lettuce	1.07±0.03a	1.19±0.05a	1.24±0.07a	0.99±0.02ab	0.45±0.06a
Cabbage	2.51±0.01a	4.12±0.02b	2.58±0.01ab	1.43±0.03ab	0.62±0.02b

Each value is mean of three replicates. Means within each column followed by the same letter are not significantly different at 5% level as determined by Duncan's multiple range test.

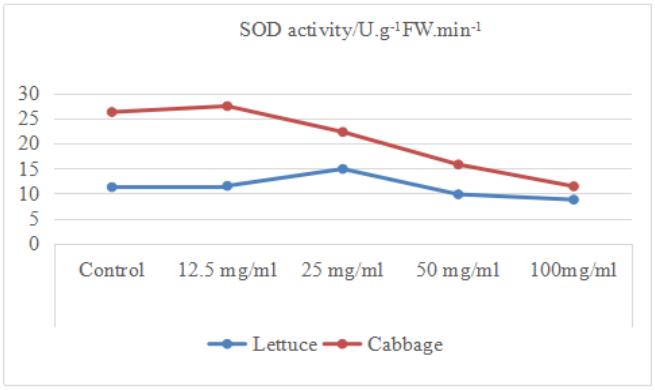

Figure 5. Effects of persimmon leaves extracts on SOD of lettuce and cabbage.

POD activity: The POD is plant protection enzyme, which removes the hydrogen peroxide from the plant, it along with SOD protects the plants against oxidative stress under adverse conditions (Fig. 5). The persimmon leaves extracts influences the activity of POD in the seedlings. In lettuce, at 12.5 mg/ml and 25 mg/ml concentration, the POD activity was similar to control, concentrations decreased the activity than control but at 25 mg/ml concentration, the POD activity began to decline, and at 100 mg/ml concentration, the POD activity decreased by 50.56% ($P < 0.05$) (Fig. 6). In cabbage, at 12.5 mg/ml concentration the activity of POD was higher than control, but was decreased at higher

concentrations. At 100 mg/ml concentrations, the POD activity decreased by 44.75% ($P<0.05$). It indicated that the allelopathic effects of persimmon leaves at high concentration and affected the POD enzymes activity and metabolism of active oxygen used, thereby inhibited the root and young seedlings growth.

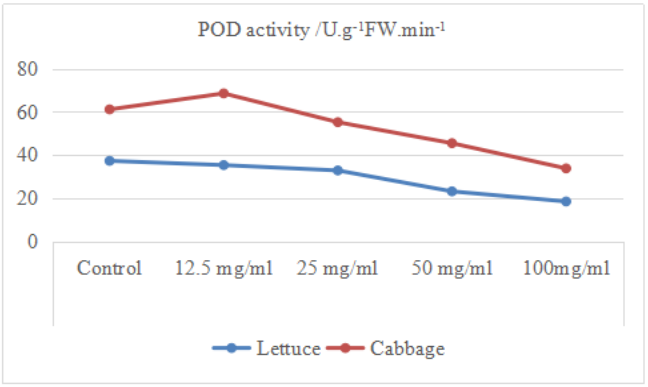

Figure 6. Effects of persimmon leaves extracts on POD of lettuce and cabbage.

CAT activity: CAT enzymes in plants decompose the H_2O_2. The decline in CAT activity increases the accumulation of H_2O_2, thereby increasing the lipid peroxidation of cell membrane (7). With the increase in concentration of persimmon leaves extract the CAT activity of receptor plant first increased and then decreased (Fig. 7). The persimmon leaves extract had concentration dependent effects on catalase activity. In lettuce, CAT activity was decreased by 8.46% and 46.6% at 12.5 and 100 mg/ml concentration, respectively. Likewise in cabbage the activity decreased by 26.4% and 73.11% at 12.5 and 100 mg/ml concentration, respectively. Thus that the extract of persimmon leaves decreased the activity of CAT in seedlings, which eliminated the H_2O_2 and increased the ability of plants to repair the cell membrane.

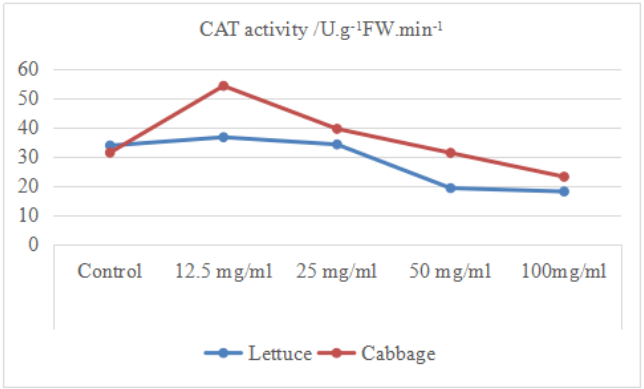

Figure 7. Effects of persimmon leaves extracts on CAT of lettuce and cabbage.

MDA activity: Malondialdehyde (MDA), a product of membrane lipid peroxidation, measures the membrane lipid peroxidation index. Membrane lipid peroxidation is initiated by oxygen free radicals, especially OH (30), which is produced by chain reaction. Therefore, the change of MDA content reflects the activity of oxygen free radicals in the plants. To a certain extent, with more accumulation of MDA, the activity of OH and oxygen free radicals may be increased. When the membrane lipid peroxidation increase the content of MDA, the cell membrane permeability increase and the degree of cell injury was increased. So the content of MDA indicate the extent of cell injury. The application of persimmon leaves extract on lettuce seedling, changed the content of MDA with increase in extract concentration. At 12.5 mg/ml concentration of ethanol extract, the MDA content was 15.54% for lettuce and 38.54% for cabbage and was higher than control at 100 mg/ml concentration, the MDA content increased to 177.20% ($P<0.05$) for lettuce and 142.91% ($P<0.05$) for cabbage than control (Fig. 8). This showed that the extract of persimmon leaves was less allelopathic at low concentration. When the concentration increased to a certain extent, the allelopathy effects of persimmon leaves extract on lettuce increased, degree of damage increased, membrane lipid peroxidation increased and thereby MDA content increased. It indicate that the extract of persimmon leaves effected the active oxygen metabolism in the plant body, lead to membrane damage, accumulated more MDA, which inhibited the seedlings growth.

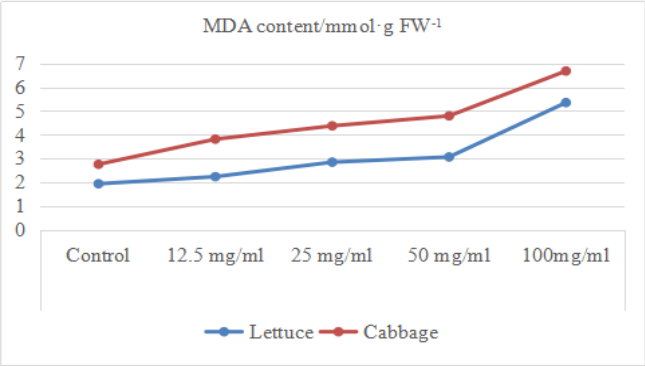

Figure 8. Effects of persimmon leaves extracts on MDA of lettuce and cabbage.

Root activity: The ability of plant tissue to restore TTC (chlorinated three phenyl nitrogen and nitrogen) indicates the degree of root growth and metabolism and to maintain the nutrients ions (14) through the energy provided by respiration. The root activity of seedlings was closely related to the concentration of persimmon leaves extract (Fig. 9), as the concentration increases the root activity increased first and then decreased. In lettuce the root activity was increased (11.21%) but decreased (57.94%) at 12.5 and 100 mg/ml concentrations, respectively, over the control. Likewise, in cabbage the root activity increased (64.4%) at 12.5 mg/ml concentrations, but decreased (35.21%) at 12.5 and 100 mg/ml concentrations over the control. It indicated that the impact of persimmon leaves extract on root activity of seedling was mainly expressed at high or low concentrations.

Allelopathy exists between numerous plants species. The allelochemicals found in plants are mainly secondary metabolites, usually with low molecular weight and simple structures and these are present in roots, stems, leaves, flowers, fruits and seeds. The most common are low molecular weight organic acids, phenolics and terpenoids, the. plants containing such numerous substances exhibits allelopathy. Persimmon leaves contain flavonoids, alkaloids, volatile oils, tannins, phenols, coumarins, triterpenes, organic acids, phytosterol, resins, and other chemicals (3), but their allelopathic effects in bioassays on surrounding plants and antimicrobial assay have been rarely reported. A previous GC-MS study showed that the main components of petroleum ether extract of dry persimmon leaf contained tannins, saccharides, steroids, flavones, cardiac glycosides, anthraquinones, naphtha and alkaloids, saponins, amino acids, polypeptides, organic acids, phenolic compounds (15). The secretion of flavone glycosides from the roots of apple trees strongly inhibits the growth of young apple trees, thereby making the survival difficult of replanted apple orchards. Hydrocyanic acid and benzoic acid are produced in the decomposition of litter residues from peach trees which are harmful to peach tree seedlings (19). Two major flavonol allelochemicals [kaempferol (H) and kaempferol-3-O-α- L-arabinofuranoside (I)] were isolated in walnut leaves extracts (5). The juglone and walnut leaf extracts drastically decreased the seed germination, seedling growth, protective enzyme systems, and the malondialdehyde content in turnip (*Brassica rapa* L.) (4).

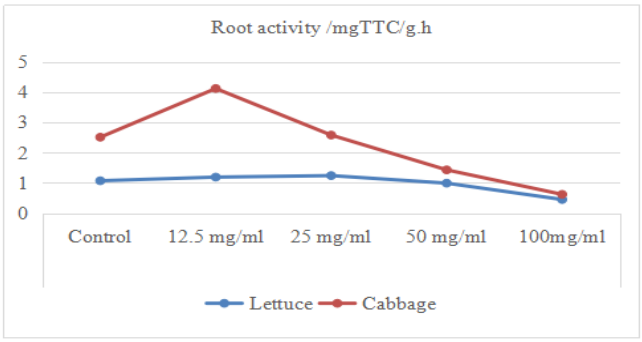

Figure 9. Effects of persimmon leaves extracts on root activity of lettuce and cabbage.

Many studies have pointed out that external stresses disturb the balance between the free radical production and removal in plants, reduce the scavenging capacity of free radical scavenging systems, which accumulate in the body, causing protein oxidation and membrane lipid damage (14, 20). Malondialdehyde (MDA) an important product of membrane lipid peroxidation, is easy to produce cross-linking of biological macromolecules, and it can make the DNA inactive or mutation and induce cell canceration, that damage the biofilm cells and cell. So MDA has been identified as the final product of lipid peroxidation, whose content is an important index (6). SOD catalyze O_2. dismutase lipid peroxidation intensity of generation H_2O_2, which is the first line of defence of plants against free radicals damage. In addition, the POD and CAT together with catalyst could affect the H_2O_2 decomposition to effectively reduce the generation of reactive oxygen species and the amount of OH. During the production of reactive oxygen

species (ROS), to remove the dynamic equilibrium state, the coordination of three protective enzymes (SOD, POD, and CAT), could effectively prevent the ROS and induce the lipid peroxidation and other damage, because single anti oxidase is not enough for the antioxidant defence of oxygen stress. Therefore, the MDA content, SOD, POD and CAT enzyme activities in plants are often more active to influence the stress level of plants (26). Our results showed that the CAT, SOD and POD in receptor plants enhanced the enzyme activity to resist the stress generation by the allelopathic substances in persimmon leaves extracts and increased the three antioxidant enzymes activities.

The activity of SOD, POD and CAT decreased with the increase concentration of aqueous extract from persimmon leaves, due to the allelopathic effects of leaves of *Persimmon sinensis*. The rice leaf significantly inhibits the activity of SOD and POD in barnyard grass and increase the MDA content (11). Likewise, the watermelon plant water extracts increases the MDA content of lettuce, with (SOD, POD, and CAT) enzyme activity.

For comprehensive evaluation of allelopathy, we should consider the effects of various factors (leaves density, litter decomposition rate, distance from other plants, and rainfall etc.) which influence the allelochemicals and their contents (25,28). Therefore, laboratory and field research methods should be combined with ecology, molecular biology, genetics, pesticide analysis, and soil science to fully elucidate the potential of allelopathy. In agricultural production, a full understanding of allelopathy may facilitate the crop rotation, intercropping, inter-planting and biological weed control, while the identified allelochemicals could be used to develop natural herbicides.

CONCLUSIONS

Extracts from persimmon leaves were tested at four concentrations (12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml) with 6-receptor plants [lettuce (*Lactuca sativa* L. *var. asparagina* Bailey), mung bean (*Vigna radiata* (L.) Wilczek), millet (*Setaria italica* (L.) Beauv. *Var. Germanica* (Mill.) Schrad, cabbage (*Brassica pekinensis* L.), rapeseed (*Brassica campestris* L.) and corn (*Zea mays* L.)], to determine their effects on seed germination, seedling growth and chlorophyll content of seedlings. The persimmon leaf extracts inhibited the seed germination and seedling growth of 6-receptor plants and the inhibitory effects were most stronger on lettuce and cabbage. Thirty seven compounds were identified in the extract of fresh persimmon leaves, the important allelochemical were: 1,2-Benzenedicarboxylic acid, P-hydroxybenzoic acid, Dibutyl phthalate, 2,4-Diethyl phthalate, n-Hexadecanoic acid, Indoleacetic acid, Octadecanoic acid, 1,2-Benzenedicarboxylic acid, Protocatechuic acid, P-hydroxybenzoic acid, Octadecanoic acid, Oleic acid, Nonadecane and Heneicosane, which may significantly affect the growth and development of plants and decrease the roots activity with the increasing extracts concentration. Of all the enzymes assayed (SOD, POD, CAT), the SOD and the POD activity cooperated to remove the reactive oxygen radicals. The allelopathy affected the activity of protective-enzymes of receptor plants and broke the membrane structure.

ACKNOWLEDGEMENTS

This research was supported by Study and Demonstration of Water Saving Agroforestry System in Loess Gully Area China (2015BAD07B050202). Many thanks are given to Ms. G.Y. Li and Mr. C.S. Cui for their help in field experiments.

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