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Full Length Research Paper

Allelopathic potential of *Jatropha curcas*

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Aqueous extracts of *Jatropha curcas* leaves and roots inhibited the growth of corn (*Zea mays* L.) and tobacco (*Nicotiana tabacum* L.). Increased inhibition by increasing the concentration of extracts suggests that the extracts may have inhibitory substance which possesses allelopathic potential. Different extracts of leaves and roots of *J. curcas* were bio-assayed and analyzed. The main allelopathic substance was determined by gas chromatography-mass spectrometry (GC-MS) data as azelaic acid. This compound inhibited the germination of *Z. mays* L. seeds at concentrations greater than 500 µg ml^{-1}, but the shoot and root growth were inhibited at concentrations greater than 100 µg ml^{-1}. The root and shoot growth were inhibited by 50% at 270 and 654 µg ml^{-1}, respectively. The percentage of azelaic acid in distilled water extracts of leaves was at least 0.94%, which indicated that azelaic acid may provide a competitive advantage to *J. curcas* in the defense mechanism by inhibiting the growth of neighboring crops.

Key words: Allelopathy, *Jatropha curcas,* growth inhibitor, azelaic acid.

INTRODUCTION

The Swiss botanist De Candolle, in 1832 suggested that crop plant exudates were responsible for an agriculture problem called soil sickness, but the term of allelopathy was first defined by Molish (1937) and improved by Rice (1984) to describe all direct positive or negative effects of a plant on another plant or on micro-organisms by the liberation of biochemical into the natural environment. Over the last two decades, a variety of plants such as *Bauhinia larsenii* L. (Chon, 2005), *Sorghum vulgare Pers.* (Zeng, 2001), *Lactuca sativa* L. (Maldonado, 2001), *Cynodon dactylon Pers.* (Loannis, 2005) and *Oryza sativa* L. (Olofsdotter, 1999), were reported allelopathic to the growth and population of neighboring or successional plants by releasing metabolites, either exudates from living plant tissues or plant residue decomposition into the soil. Some of these metabolites known as allelochemicals play an important role in chemical interactions in natural plant communities and the growth of neighboring or successional plants are inhibited in certain concentration of allelochemicals (Inderjit, 1996; Narwal, 1999; Putnam and Tang, 1986; Einhellig, 1996; Seigler, 1996; Dayan, 2000). Many substances were isolated and determined as the allelochemicals. For instance, ferulic, vanillic and syringic acids were identified as allelopathic substances in sugarcane straw (Sampietro et al., 2005; Sampietro and Vattuone, 2006); 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one was isolated from root exudates of wheat (Wu, 2001) and p-coumaric acid was isolated from buffalograss (*Buchloe dactyloides*) and was also detected to have allelopathic effects on the growth of annual Poa annua and buchloe dactyloides seedlings (Wu, 1998).

Jatropha curcas L. is well known as an oil-producing crop which has been cultivated widely, especially in many tropical and subtropical areas of Africa, America and Asia (Heller, 1996). *J. curcas* contains many toxicants, such as the jatrophine, curcin and phorbol esters which make the plant unpalatable and toxic to many insects and animals (Naengchomnong, 1986; Makkar, 1997; Liu, 1997; Wink, 1997). However, research articles about its allelopathic effects on other crops have been rarely reported. Considering its growth characteristics, *J. curcas* has to be cultivated with other tropical or subtropical crops, which makes the allelopathy research of *J. curcas* imperative. In this study, the allelopathic potential of *J. curcas* and its inhibitory substance were determined. The concentrations required for 50% inhibition in the roots

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and shoot of *Zea mays* L. that is, one of the most important tropical crop, were also studied.

MATERIALS AND METHODS

Leaves were collected from one year old *J. curcas* and air dried. Seeds of *J. curcas*, *Z. mays* L. (Corn) and *Nicotiana* tabacco *L.* (tobacco) were provided by the Key Laboratory of Bio-Resources and Eco-Environment in Sichuan University.

Seeds of J. curcas which were disinfected by treating with 70% ethanol solution for 1 min and 0.3% $KMnO_4$ solution for 10 min were rinsed four times with distilled water and soaked in 30°C distilled water for 48 h. Imbibed seeds were incubated at trays containing clean quartz sands in a growth chamber at 27±1°C with light intensity of 400 $\mu mol.m^{-2}.s^{-1}$ and a 16 h photoperiod for one month. Root systems from 30-day-old seedlings were air dried and weighed.

Extraction and bioassay

Preparation of root exudates

After disinfecting by 70% ethanol solution for 1 min and 0.3% $KMnO_4$ solution for 10 min and rinsing four times with distilled water, seeds of *J. curcas* were soaked in 30°C distilled water for 48 h. Imbibed seeds germinated in the wet clean quartz sands at 27±1°C with an intensity of 400 $\mu mol.m^{-2}.s^{-1}$ and a 16 h photoperiod. The method described by Muniru (2003) was used for this study. Eight-day-old plant seedlings were transferred to an aluminium sheet (42 cm × 75 cm, perforated with 3 mm diameter holes, 1 cm apart) and placed on a Perspex tray (40 cm × 70 cm × 6 cm) containing 12.5 L of distilled water. The roots of the seeds emerged from the bottom side of the tray into the water. Considering the xeric characteristic of *J. curcas*, a small air pump was used to ventilate the water to prevent the seedling roots from rotting due to lack of oxygen. Trays of *J. curcas* seedlings were alternated between distilled water and nutrient Hoagland media (Hewitt, 1966) every 3-day. Root exudates were collected from the distilled water phase and stored at less than 4°C until bioassay. A treatment of distilled water was used as the control.

Preparation of aqueous extracts

Roots and leaves were washed, clipped into small pieces (<1 cm) and air-dried for 48 h at room temperature. Materials were soaked in different quantities of distilled water to access different concentrations including 0.25, 0.1, 0.025, 0.005 and 0.0025 g ml^{-1} at room temperature (shaking every 4 h) for two days, respectively. The solutions were filtered through four layered cheesecloth to remove fiber fragment and centrifuged at 3000 rpm for 5 min to remove fiber fragment. The supernatants were then filtered through one layer of filter paper (Whatman no. 42). Extracts were stored at less than 4°C until bioassay. A treatment of distilled water was set as the control.

Preparation of different extracts of plant materials

Extracts were prepared by soaking weighed amounts of dried plant material (2 g) for 7 days in 100 ml distilled water, menthol, 50% menthol, acetic ether, acetone and dichloromethane, respectively. The extracts were filtered, centrifuged and filtered again as mentioned earlier. 10 ml of the filtrate was added to each Petri dish (9 cm diameter) containing 15 g of clean quartz sands covered with a circle (7 cm diameter) of Whatman no.1 filter paper. A control was also set using distilled water. All solvents of extracts were evaporated in the draft chamber and the filter paper in the dishes was then moistened with 10 ml distilled water to determine the allelopathic potential of different extracts.

Preparation of azelaic acids solutions of different concentrations

Standard of azelaic acid was obtained from Sigma chemical company. The treatment solutions used for the seedling growth test comparisons were prepared using 100, 200, 300, 400, 500, 600, 700, 800, 900, 1 and 2 mg ml^{-1} of the azelaic acid dissolved in distilled water. A treatment of distilled water without azelaic acid was used as the control.

Bioassay

10 ml solution of each type was added to different Petri dishes containing 15 g of clean quartz sands covered with a circle of filter paper. Ten seeds of *Z. mays* L. or 30 seeds of *N. tabacum* L. were placed in each prepared dish. Four dishes of each treatment were arranged in a completely randomized design and incubated in a growth chamber at 27±1°C, 400 $\mu mol\ m^{-2}\ s^{-1}$ intensity and 16 h photoperiod for 7 days. Germination rate was measured on the third day. Root and shoot growth of each seedling were measured on the 7th day.

For assay of *Z. mays* L. seedlings, seeds were germinated in trays containing clean quartz sands covered with filter papers in a growth chamber. Four days after germination, seedlings with similar status (4 cm root and 2 cm shoot) were selected to be treated by azelaic acid solutions of different concentrations to determine the allelopathic potential of azelaic acid, treatment and data collection as described earlier.

Imbibitions effects of Zea mays L. seed

After disinfecting and rinsing by the method described earlier, *Z. mays* L. seeds were first weighted and then soaked in aqueous extracts of leaves with different concentrations at 27±1°C for 24 h and weighted at every 4 h regular interval (dried before weighting). Seeds soaking in distilled water were set as the control at the same time. Every treatment was repeated four times.

Root reduction activities

The 2, 3, 5-triphenyl tetrazolium chloride (TTC) method (Zhang, 1990) was used to measure the root reduction activity. 0.5 g roots were taken from seedlings and washed. After blotter-drying, the roots were soaked in a 10 ml mixture (5 ml of 4 gl^{-1} TTC and 5 ml of a phosphate buffer at pH 7.0) in darkness at 37°C for 1 h. 2 ml of 1 M sulfuric acid was added to stop the reaction. Then roots were taken out, washed with deionized water, blotter-dried and mashed in a mortar with less than 3 ml ethyl acetate ($C_4H_8O_2$). The extract was filtered and put into a 10 ml tube. The total volume of the extract was added up to 10 ml and optical density of the formazen extract was obtained at 485 nm on the UV/VIS spectrophotometer (SP8000B). An enzyme unit was defined as the quantity (mg) of tetrazolium chloride reduced per hour on a per gram root (fresh weight) basis.

Chlorophyll content and physical index

The *Z. mays* L. seedlings, treated with aqueous extracts or root

exudates of *J. curcas* at 27±1℃ and grown under photon intensity of 400 μmol $m^{-2}s^{-1}$ artificial light and a 16 h photoperiod for 10 days, were used to measure leaves photosynthesis rate, stomatal conductance, transpiration rate and substomatal CO_2 concentration. These photosynthetic indexes were detected using LI-6200 portable photosynthesis system (Li-Cor Inc., Lincoln, NE, USA) at 400 μmol photons $m^{-2}s^{-1}$ and 27±1℃.

After photosynthesis measurement, *Z. mays* L. leaves were harvested for chlorophyll (Chl) determination. Half gram leaves were placed in a 10 ml mixture of acetone (C_3H_6O), anhydrous ethanol and water (45:45:10 V/V) for 48 h in the dark at 4 to 5℃. The absorption of each Chl extract was read using the UV/VIS spectrophotometer (SP8000B) at 665 and 649 nm; Chla, Chlb and total Chl contents were calculated by the Arnon formula (Arnon, 1949).

Esterification and gas chromatographic analysis

GC-MS analysis was carried out on a mixed standard of the compounds of interest and representative plant samples to confirm the identity of component peaks. Before the analysis, esterification was used to increase the volatility of the compounds. Standard chemical compounds were purchased from Sigma chemical company. The method described by Finney (2005) was used for this study. All the samples were reduced to dryness under a N_2 stream at 40℃. 400 μl of MSTFA–DMF (1:1) were added to the sample, the samples were capped under N_2 and then heated at 75℃ for 30 min. Samples were then used for GC analysis.

GC-MS analysis of different extracts-GC-MS analysis was performed with GCMS-QP2010 Plus (Shimadzua Co.) and a DB-1HT 30 m×0.32 mm×0.1 μm column was used for this study. The GC temperature program was initially set to 130℃, raised to 180℃ at a rate of 10℃.min^{-1} and then held at this temperature for 5 min; then raised from 180 to 220℃ at a rate of 5℃ min^{-1} and held at this temperature for 2 min; raised from 220 to 250℃ at a rate of 30℃ min^{-1} and held at this temperature for 15 min. Ultra-high purity helium was used as carrier at a flow rate of 1.0 ml min^{-1}. The inlet (splitless), GC interfaces and ion chamber temperatures were 270, 250 and 200℃, respectively. The sample injection volume used was 2.0 μl.

Data analysis

The data averaged by 3 times repetitions from each experiment were subjected to an analysis of variance, with the significant differences among means identified by Duncan's multiple range test ($P < 0.05$). Dose-response curves were constructed to determine the concentration of azelaic acid required for 50% inhibition of *Z. mays* L. germination, root and shoot growth of seedlings.

RESULTS

J. curcas is a kind of drought-tolerant plant which root is easy to get rotten because of high moisture. A pump was used to pump air into the medium to make seedlings of *J. curcas* grow well and collect root exudates successfully. The effect of root exudates (3.2 strains.l^{-1}) on germination and seedling growth of *N. tabacum* L. and *Z. mays* L. were studied and the results showed that the exudates had significant inhibitory effect on germination and seedling growth of both *N. tabacum* L. and *Z. mays* L., especially on the root growth of seedlings (Table 1). It also had significant effect on physiological index. The exudates apparently decreased the photosynthetic rate, stomatal conductance and substomatal CO_2 concentration, but significantly increased the transpiration rate (Table 1). The root exudates decreased chlorophyll a and b, but increased the ratio of Chla/b.

Imbibitions as the prerequisite of germination were studied to find out whether it was affected by the aqueous extracts of leaves. The results showed that the whole imbibitions was divided into three phases (Figure 1): in the first 2 h, (P1) after *Z. mays* L. seeds were immersed in the aqueous extracts or distilled water, the absorbing rate of seeds was high and showed no differences; from 2 to 4 h, (P2) the seeds absorbed water more rapidly than P1 and there began to show differences in between the treatments of different concentrations; in the last phase (P3), the absorbing rate was much smaller than P1 and also showed significant differences between each treatment. The aqueous extracts of leaves mainly disturbed the imbibitions of *Z. mays* L. seeds in P2 and P3 period. In contrast to the control, lower concentrations of aqueous extracts (<0.005 g.ml^{-1}) stimulated absorbing rate of *Z. mays* L. seeds, while higher concentrations of aqueous extracts (>0.025 g.ml^{-1}) inhibited the absorbing rate (Figure 1).

As shown in Figure 2, the aqueous extracts of leaves and roots did not adversely affect the germination and root growth of *N. tabacum* L. and *Z. mays* L. at a low concentration, that is, < 0.0025 g ml^{-1}. However, the extracts of high concentration significantly inhibited the germination and root growth of *N. tabacum* L. and *Z. mays* L. In terms of species, the aqueous extracts at the low concentration, which promoted the seed germination and seedling growth of *Z. mays* L., had little effect on *N. tabacum* L. The inhibitory effects increased with increasing concentrations. However, aqueous extracts of different parts presented different inhibitory effects; the inhibitory degree of leaves was higher than that of roots (Figure 2).

Photosynthesis, in terms of photosynthetic rate, stomatal conductance and intercellular CO_2 concentration had the similar effects of aqueous extracts on germination and root growth, that is, not affected at low concentrations, but inhibited at high concentrations and the inhibitory effect enhanced with increasing concentration. Similarly, aqueous extracts of leaves also showed a stronger inhibition (Figure 3a, c, d). On the contrary, the transpiration rate got increased as the concentration of aqueous extracts elevated. The aqueous extracts of leaves enhanced the transpiration rate of *Z. mays* L. leaves more than that of roots (Figure 3b).

Similarly, low concentrations (<0.0025 g.ml^{-1}) enhanced the root growth of *Z. mays* L. seedlings and high concentrations (>0.005 g.ml^{-1}) decreased it. The aqueous extracts of leaves also showed a stronger inhibitory effect (Figure 4).

Table 1. The effects of root exudates at 3.2 strains.l^{-1}on germination and root growth of *N. tabacco L.* and *Z. mays* L.

Plant	Parameter	Treatment	
		0 (Control)	Root exudate
Nicotiana tabacum L.	Germination rate (%)	90	84*(-0.06)
	Radicle length (cm)	1.82	0.423**(-0.76)
Zea mays L.	Germination rate (%)	59	48*(-0.114)
	Radicle length (cm)	17.2	11.7**(-0.319)
	Root activity ($\mu gg^{-1\ -1}$)	1221	830**(-0.32)
	Photosynthetic rate ($\mu mol\ CO_2.m^{-2}.s^{-1}$)	32	28*(-0.125)
	Transpiration rate ($mmol\ H_20.m^{-2}.s^{-1}$)	4.2	7.5**(0.44)
	Stomatal conductance ($mol\ H_20.m^{-2}.s^{-1}$)	0.2	0.16*(-0.2)
	Substomatal CO_2 concentration ($\mu l.l^{-1}$)	155	117**(-0.245)
	The total chlorophyll content ($mg.g^{-1}$)	1.603	1.136**(-0.291)
	Chlorophyll a content ($mg.g^{-1}$)	1.239	0.964**(-0.221)
	Chlorophyll b content ($mg.g^{-1}$)	0.364	0.172**(-0.52)
	Chl a/b	3.4	5.6**(0.392)

* Significant difference at the ≤ 0.05 level;** significant difference at the ≤ 0.01 level.

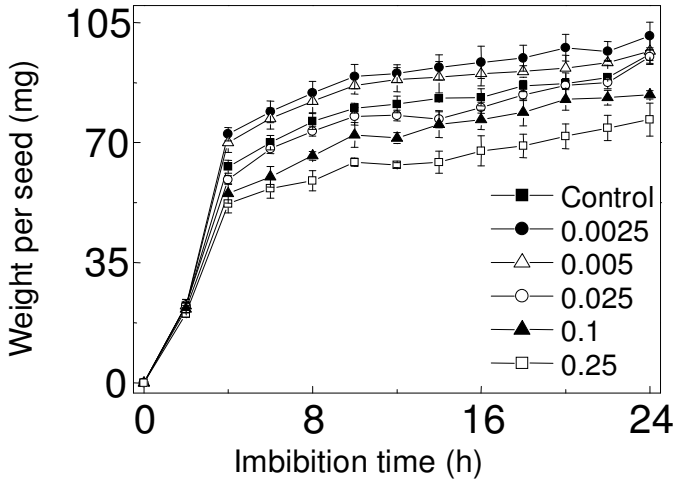

Figure 1. Imbibition rate of *Z. mays* L. seed in extracts of leaves.

The chlorophyll content which as an important physiological indicator of plants had been studied to detect the allelopathic potential of aqueous extracts on photosynthesis. Chlorophyll b content was apparently inhibited by the aqueous extracts of leaves. The inhibition increased abruptly till the concentration of used extract reached 0.005 $g.ml^{-1}$ and this inhibition trend became mild when concentration of extract was over 0.005 $g.ml^{-1}$ (Figure 5). On the contrary, aqueous extracts of leaves at low concentration (<0.0025 $g.ml^{-1}$) rapidly stimulated the content of Chl a (Figure 5). Therefore, the contradictory effects made Chl a/b much higher than control. In seedlings grown in 0.0025 $g.ml^{-1}$ Chl a/b was 6.2 against 3.3 in control. However, as the decreasing of total content of chlorophyll, the Chla a/b decreased, while the concentration of aqueous extracts increased.

Acetic ether, 50% menthol, menthol, dichloromethane, distilled water and acetone extracts of leaves inhibited not only the germination of *Z. mays* L. seeds, but also root and shoot growth of *Z. mays* L. seedlings (Figure 6). The different extracts obtained from 2 g dried leaves of *J. curcas* as in the order given earlier, inhibited the germination of *Z. mays* L. seeds respectively, to 3.8, 34.9, 45, 6.6, 19.9 and 0% of control germination rate and also inhibited the root growth of *Z. mays* L. seedlings to -8.9, 79.3, 91.8, 18.2, 44.3 and 13.4%, respectively of control root growth. The different extracts of leaves also inhibited the shoot growth, respectively to 2.6, 47.6, 53.2, 19.7, 34.9 and 7.3% of control shoot growth. The 50% menthol and menthol extracts performed relatively more

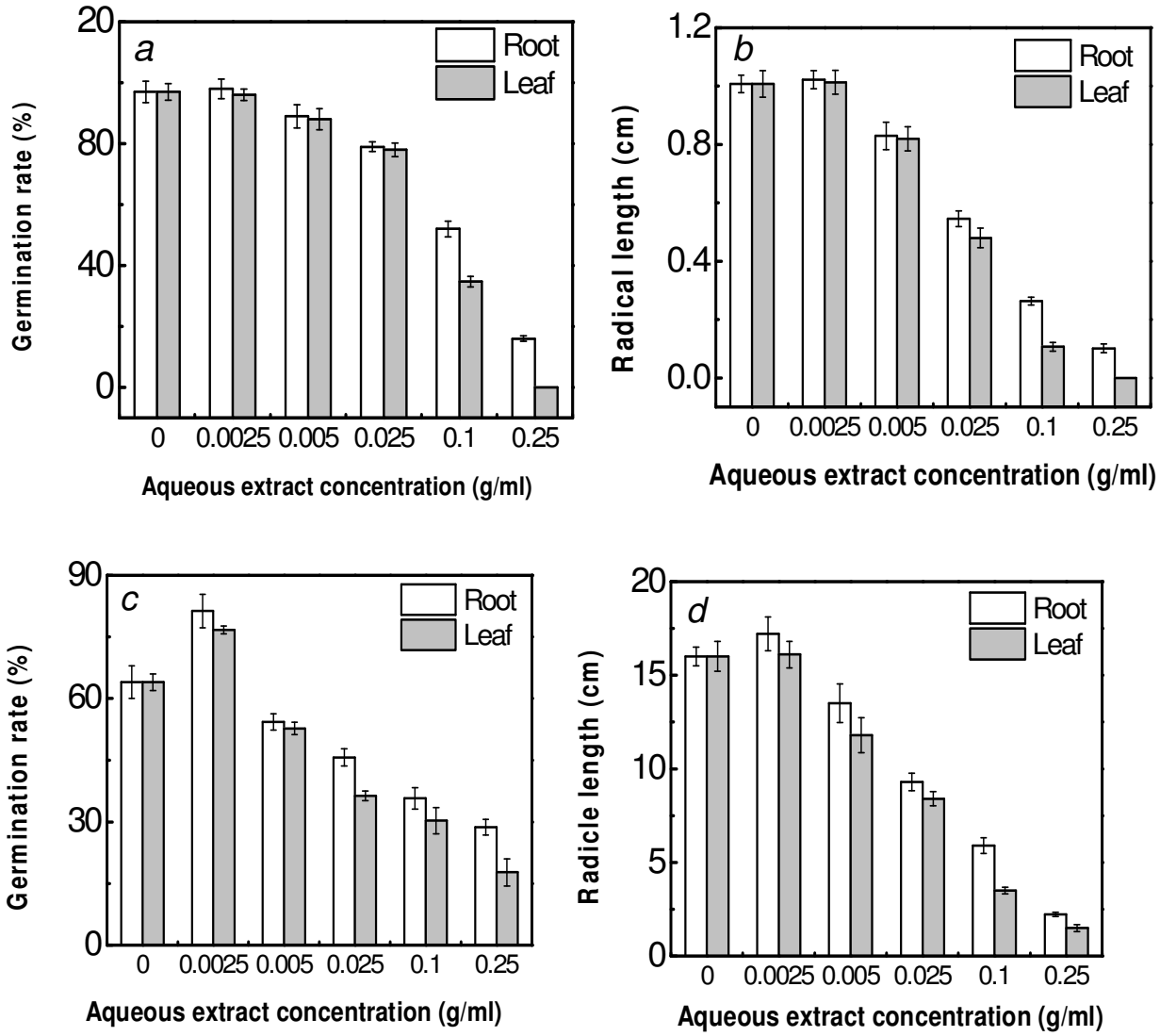

Figure 2. Effects of differently concentrated aqueous extracts of *J. curcas* on; (a) Germination rate of *N. tabacum* L. seeds; (b) root length of *N. tabacum* L. seedlings; (c) germination rate of *Z. mays* L. seeds; (d) root length of *Z. mays* L. seedlings.

significant inhibitory effects on germination and seedling growth of *Z. mays* L., in contrast the acetic ether extracts showed a slight promotion on root growth of corn seedlings.

Similar to the result obtained by extracts of leaves, different extracts of roots inhibited both the seeds germination and seedlings growth of *Z. mays* L. The different extracts obtained from 2 g dried roots of *J. curcas* inhibited the germination of *Z. mays* L. seeds, respectively to 3.8, 19.9, 39, 10, 30 and 6.6% of the control germination rate and root growth of *Z. mays* L. seedlings , respectively to -5.5, 18.7, 89.4, 18.1, 18.3 and 5.2% of the control root growth. The different extracts of leaves also inhibited the shoot growth, respectively to 2.5, 26.6, 45.9, 11.5, 24.3 and -4% of the control shoot growth. Compared Compared with the other extracts, the menthol extract showed a more significant inhibition than other treatments (Figure 6). Acetic ether and acetone extracts of root also showed a slight promotion effects, respectively on root and shoot growth of *Z. mays* L. seedlings.

GC-MS analysis showed that the main substances constituting the extracts of leaves and roots were azelaic acid, oleic acid, hexadecanoic acid and octadecadienoic acid, which all showed a relatively consistent tissue concentration. But menthol, 50% menthol and aqueous extracts all contained azelaic acid or had a higher concentration of it than other extracts (Table 2); this was consistent with the results in Figure 6. Therefore, azelaic acid was chosen for the following study to detect its allelopathic potential. The GC-MS spectrum of azelaic acid was shown in Figure 7.

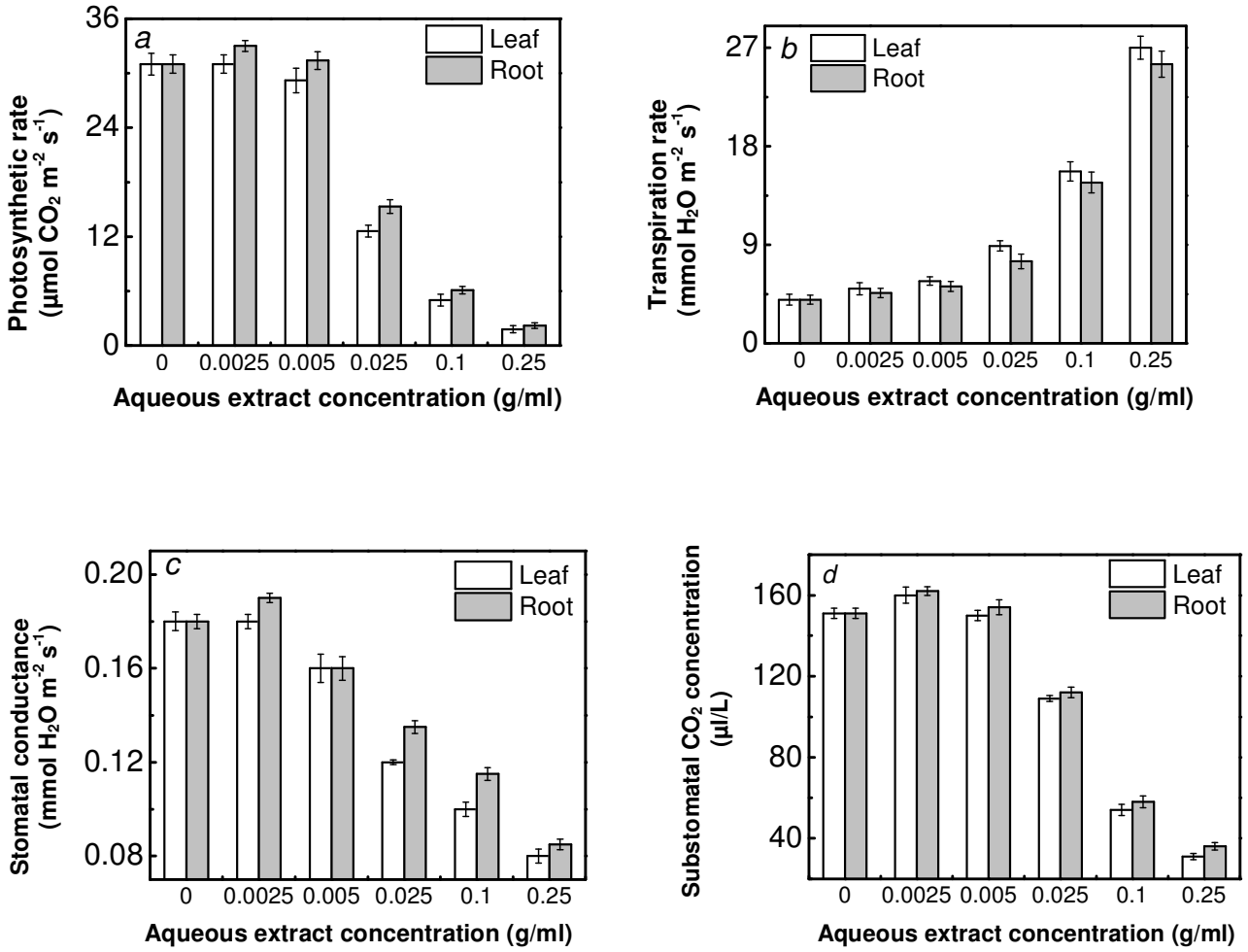

Figure 3. Effects of *J. curcas* aqueous extracts on (a) photosynthetic rate; (b) transpiration rate; (c) stomatal. conductance; (d) substomatal CO_2 concentration of *Z. mays* L. seedlings.

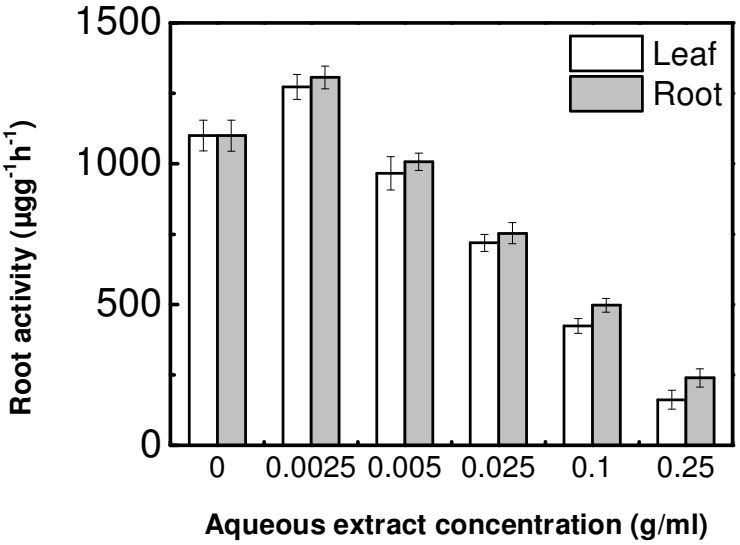

Figure 4. Effects of *J. curcas* aqueous extracts on root vigour of *Z. mays* L. seedlings.

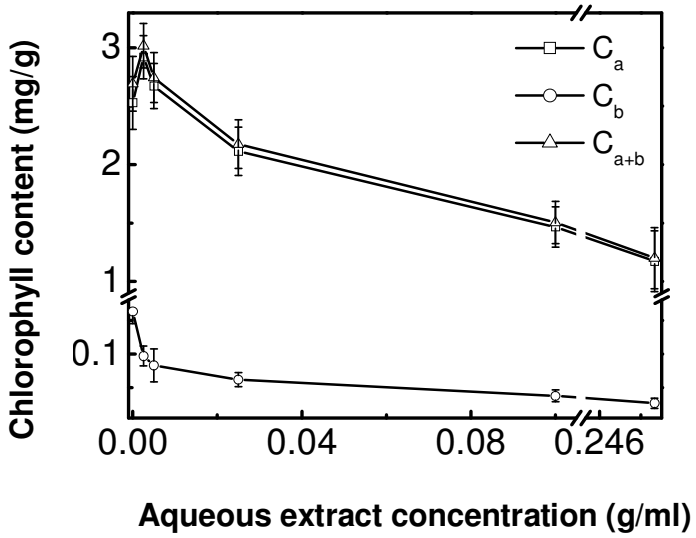

Figure 5. The effect of *J. curcas* aqueous extracts on chlorophyll of *Z. mays* L. seedling leaves.

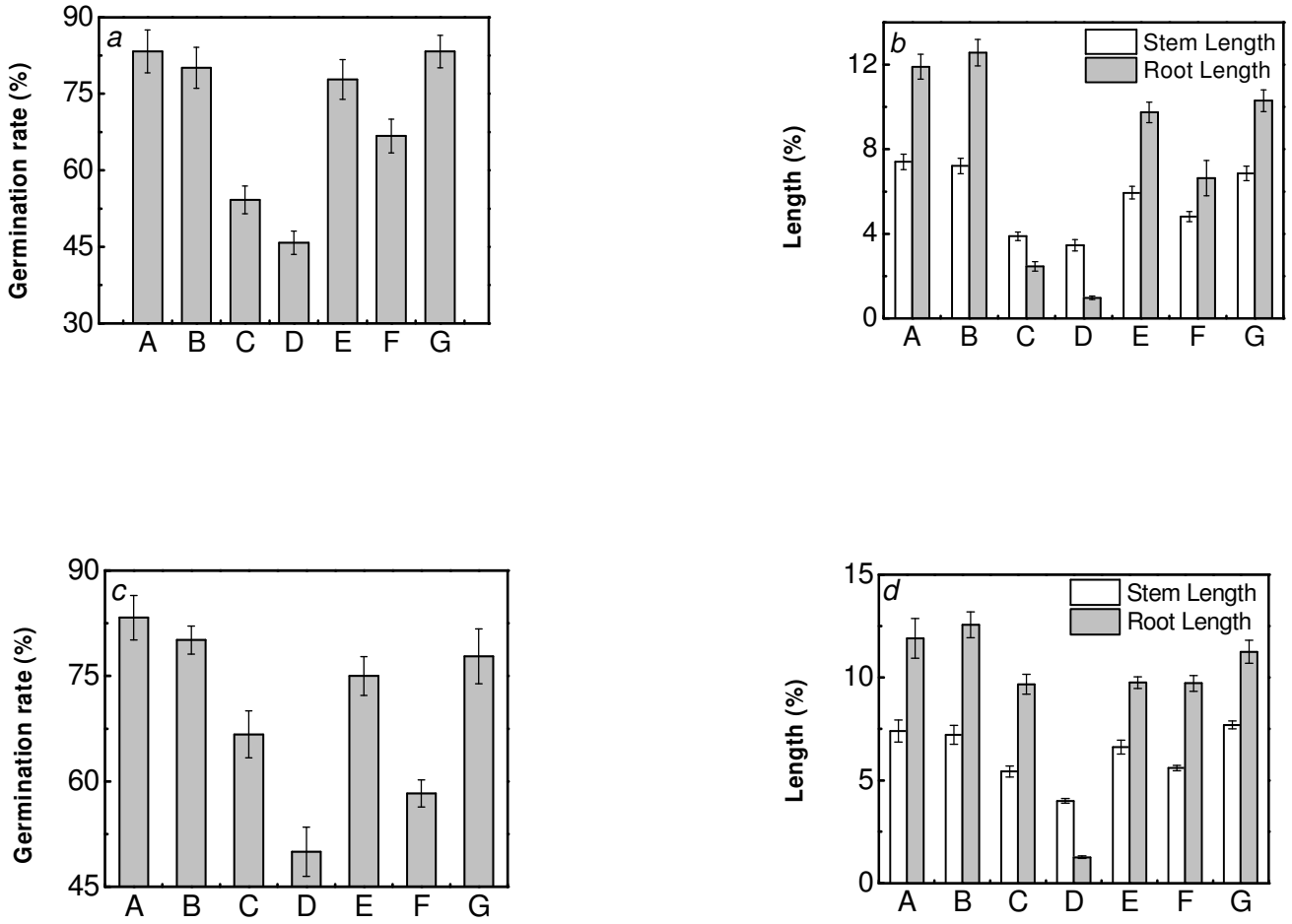

Figure 6. Resistance of *Z. mays* L. to different, A= control; B= acetic ether; C= 50% menthol; D= menthol; E= dichloromethane; F= distilled water; G= acetone; extracts of *J. curcas* In terms of: (a) germination rate of seeds treated by different extracts of leaves; (b) stem and root length of seedlings treated by different extracts of leaves; (c) germination rate of seeds treated by different extracts of roots; (d) stem and root length of seedlings treated by different extracts of roots.

Table 2. Contents of main substances in different extracts.

Main substance	Dichloromethane (%)	50% menthol (%)	Menthol (%)	Distilled water (%)	Acetic ether	Acetone (%)
Root						
Azelaic acid	0.31	0.6	8.47	0.73	Not found	Not found
Oleic acid	55.01	25.74	42.49	5.25	Not found	Not found
Hexadeca-noic acid	13.73	11.35	11.82	Not found	Not found	5.74
Octadecan-oic acid	11.01	16.67	13.29	Not found	Not found	Not found
Leaf						
Azelaic acid	Not found	1.83	10.56	0.94	Not found	Not found
Oleic acid	52.87	26.73	52.98	6.64	58.33	29.99
Hexadeca-noic acid	12.12	Not found	14.74	1.23	Not found	0.1
Octadecan-oic acid	16.03	9.02	16.58	1.02	4.46	16.94

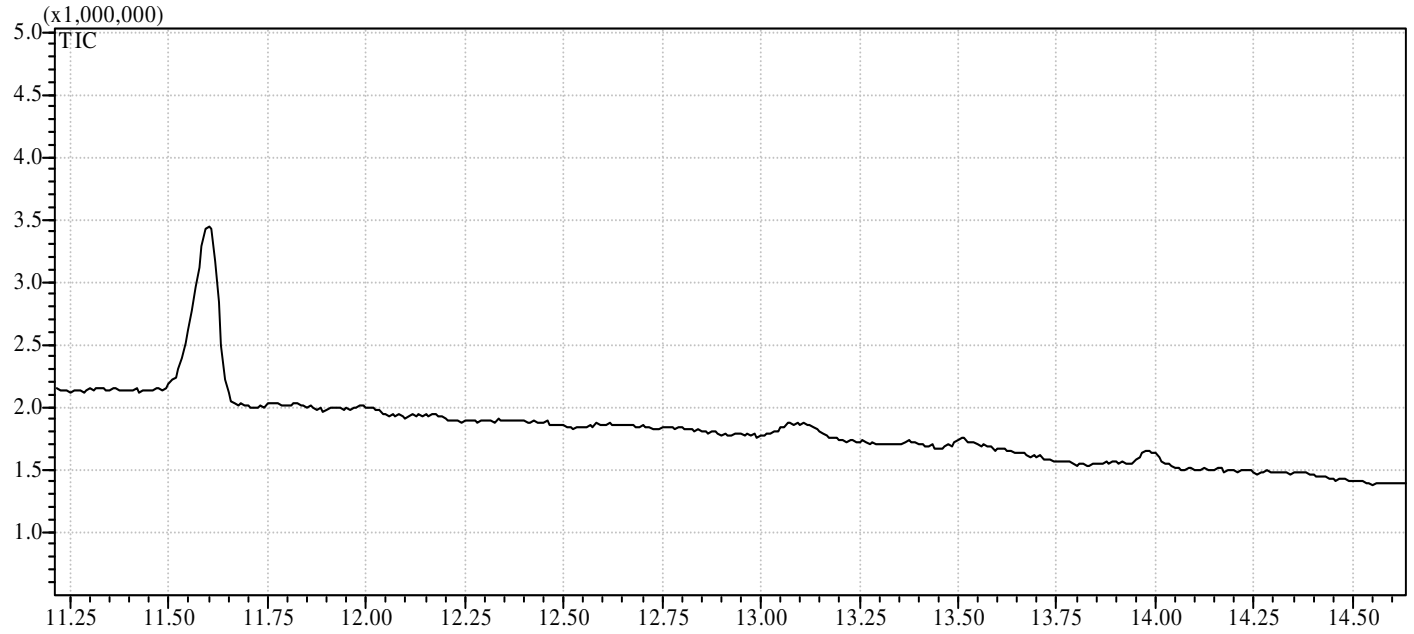

Figure 7. Azelaic acid in GC-MS analysis.

Considering that azelaic acid might be a possible pollutant during GC-MS analysis, we analyzed all the solvents without samples under the same conditions. Subsequently, no azelaic acid was detected in all the solvents. When the concentration of azelaic acid was below 500 μg ml^{-1}, it showed little inhibitory effects on the germination of *Z. mays* L. seeds, but inhibited the shoot and root growth of seedlings more significantly (Figure 8a, b). The inhibition increased with increase in the concentration. As the interference to growth of *Z. mays* L. seeds, azelaic acid also showed strong inhibition on *Z. mays* L. seedlings which had germinated for 4 days before been treated with azelaic acid solutions. Compared with the shoot, azelaic acid inhibited root growth of seedlings more significantly and this inhibition also increased with increasing concentration (Figure 8c).

Germination inhibition (R^2=0.84).

$$y = 46.26451 - \frac{59.26925}{1+\exp(2.61154x - 12.58611)}$$

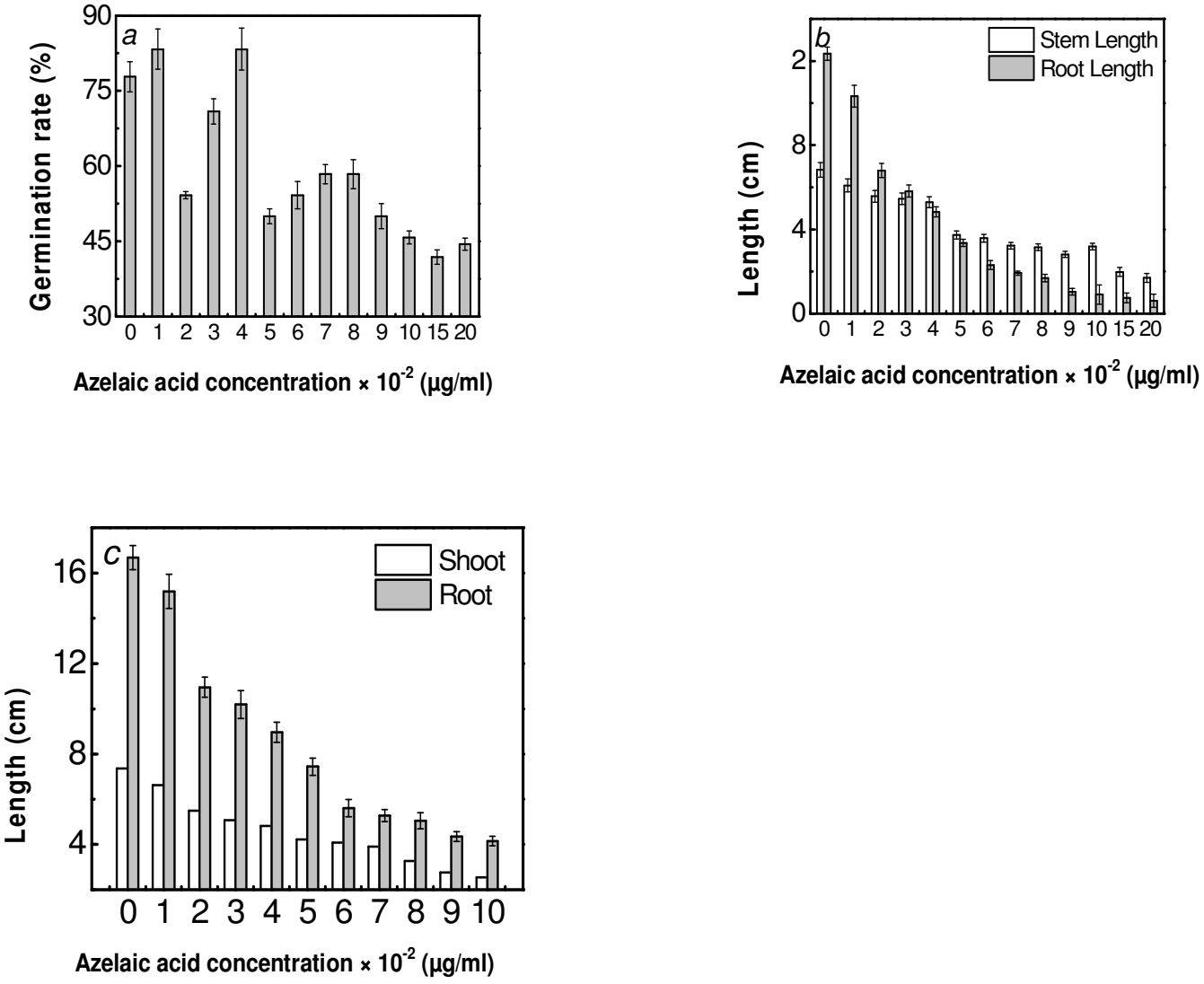

Figure 8. Response of *Z. mays* L. to different concentrations of azelaic acid solutions in terms of: (a) germination rate of seeds; (b) stem and root length; (c) stem and root length of *Z. mays* L. seedlings which had germinated for for days before been treated with azelaic acid solutions.

Root growth inhibition (R^2=0.97)

$$y = 95.05703 - \frac{85.5022}{1+\exp(1.73708x\text{-}5.94581)}$$

Shoot growth inhibition (R^2=0.97)

$$y = 76.41938 - \frac{161.16738}{1+\exp(0.21100x - 0.11040)}$$

In which R is the correlation coefficient.

Statistically, Boltzmann equations were fitted to these data (Figure 9). Germination of *Z. mays* L. decreased as the concentration increased (Figure 9). The concentration required for 50% germination rate of *Z. mays* L. was 970 µg ml^{-1}. In contrast, root and shoot growth were inhibited by 50% at 270 and 654 µg ml^{-1}, respectively. R^2 of the germination inhibition was 0.84 which indicated that the interference of azelaic acid solution on germination was not as regular as it was on root and shoot growth of the seedlings.

DISCUSSION

Corn and tobacco, which are the two major crops widely co-cultured in the tropical and subtropical areas of Africa, America and Asia, were used to test the allelopathic potential of *J. curcas* on other plants in our research. Our

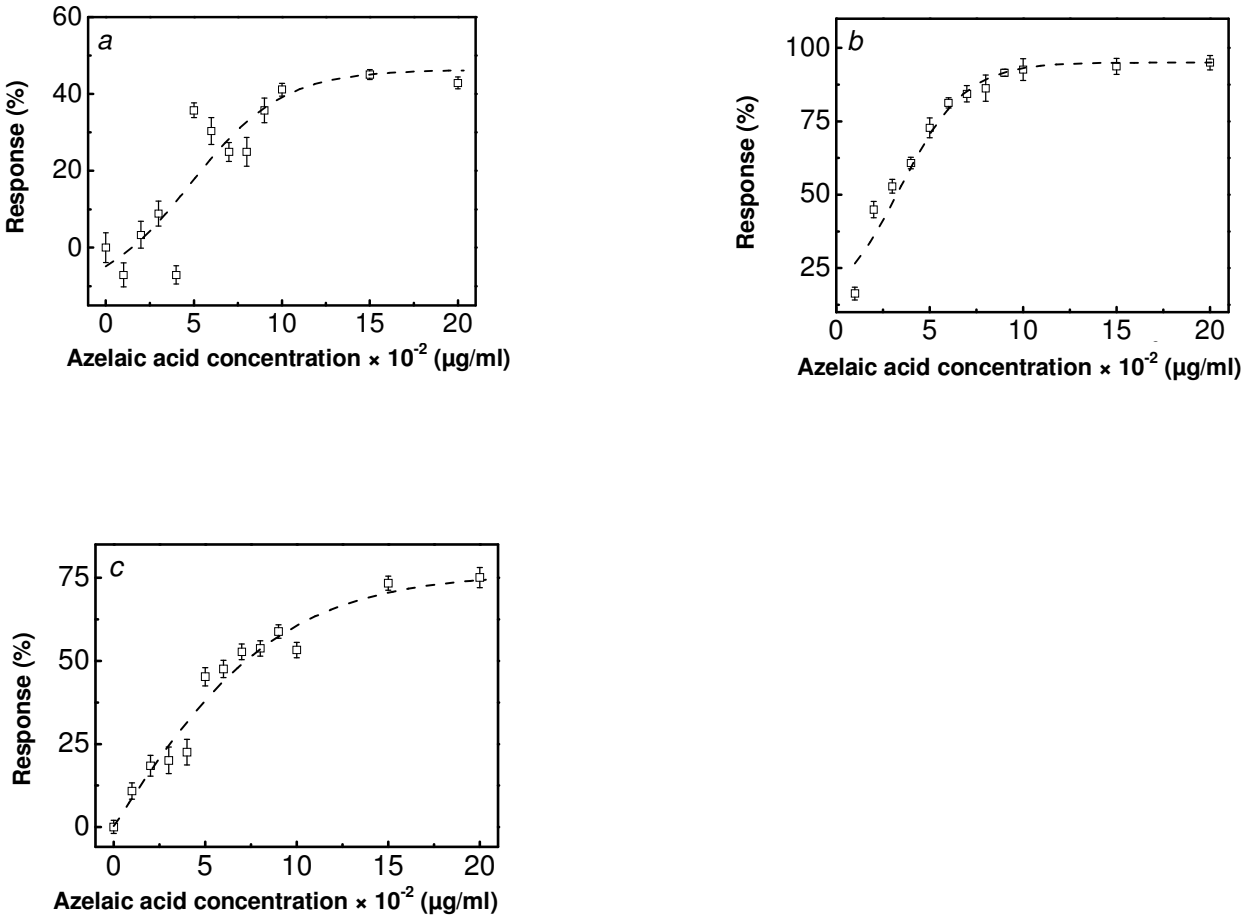

Figure 9. Dose-response curves for (a) germination; (b) root growth; (c) shoot growth of *Z. mays* L. to azelaic acid solutions.

results showed that aqueous extracts of *J. curcas* at low concentration had little effect on seed germination and seedling growth, while high concentration inhibited these processes. However, at higher concentrations, the stomatal conductance of *Z. mays* L. leaves was inhibited by aqueous extracts significantly, which led to the increase of stomatal resistance and decrease of intercellular CO_2 concentration. Photosynthetic rates and chlorophyll content of the leaves also declined by inhibiting the synthesis and degradation of chlorophyll. Additionally, the intense enhancement of transpiration resulted in the dehydration of leaves. Naturally, inadequate solar energy entrapment from photosynthesis which had been inhibited and more consumption led to decline growth and even death of the *Z. mays* L. seedlings. Besides, the aqueous extracts and root exudates also inhibited the root growth of *Z. mays* L. seedlings by reducing root activity.

In terms of the physiological point, the growth of crops was affected by aqueous extracts in various ways. The water absorption of the seeds were presented in two ways; passive and active absorption, as Kramer mentioned in 1940 (Kramer, 1940). In our research, aqueous extracts of *J. curcas* significantly decreased active absorption may be caused by inhibiting the metabolic activity of *Z. mays* L. seeds, while the passive absorption which was mainly manipulated by the permeability of water to the seeds was not affected that much (Figure 1).

In order to further our study, extracts made by different solvents were bio-assayed and analyzed by GC-MS. Menthol, 50% menthol and aqueous extracts of leaves and roots showed an inhibitory effect that was more significant than the other solvents. Besides, the results of GC-MS analysis showed that menthol, 50% menthol and aqueous extracts all contained azelaic acid or had a higher concentration of it than other extracts which was consistent with the inhibitory effect of these extracts. Hence, azelaic acid was chosen for the following study. The initial inhibitory concentration of azelaic acid solution on growth of *Z. mays* L. seedlings was 100 µg ml^{-1} or less (Figure 8). As the results calculated by the Boltzmann equations earlier mentioned, the concentrations required for 50% inhibition in the root and shoot growth of *Z. mays* L. seedlings were 270 and 654 µg ml^{-1}, respectively. Combined with the results of GC-MS, the percentage of

azelaic acid in the distilled water extracts of the leaves was at least 0.94%. Considering the endogenous level and the inhibitory activity, this compound may provide a competitive advantage over other crops as it may be involved in the defense mechanism through the inhibition of the growth of neighboring crops. In addition, azelaic acid, which has also been found in other plants including wheat and sorghum *bicolor* (Sun and Sun, 2001; Mehnood et al., 2008) may contribute to the inhibitory effect of the plant.

REFERENCES

Arnon DI (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24: 1-15.

Chon SU (2005). Allelopathic potential in lettuce (*Lactuca sativa* L.) plants. Sci. Horicult. 106: 309-317.

Dayan FE, Romagni JG, Duke SO (2000). Investigating the mode of action of natural phytotoxins. J. Chem. Ecol. 26: 2079–2094.

Einhellig FA (1996). Interactions involving allelopathy in cropping systems. Agron. J. 88: 886–893.

Finney MM, Danehower DA, Burton JD (2005). Gas chromatographic method for the analysis of allelopathic natural products in rye (*Secale cereale* L.), J. Chromatography A. 1066: 249-253'

Heller J (1996) Physic nut *Jatropha curcas* L. Promoting the conservation and use of underutilized and neglected crops. Institute of Plant Genetics and Crop Plant Research, Gatersleben: Inter. Plant Gene. Res. Instit. Rome Italy, p. 66.

Hewitt EJ (1966). Sand and water culture methods used in the study of plant nutrition. 2nd Ed. Bucks, UK. Commonwealth Agric. Bureaus.

Inderjit (1996). Plant phenolics in allelopathy. Bot. Rev. 62: 186–202.

Kramer PJ (1940). Causes of Decreased Absorption of Water by Plants in Poorly Aerated Media. Am. J. Bot. 27: 216-220.

Liu L, Sporer F, Wink M, Jourdane J, Hennig R, Li YL, Ruppel A (1997) Anthraquinones in *Rheum palmatum* and Rumex dentatus (Polygonaceae) and phorbolesters from J. *curcas* (Euphorbiaceae) with molluscicidal activity against schistosomias vector snails Oncomelania, Biomphalaria and Bulinus. Tropical Med. Int. Health, 2: 179–188.

Loannis V, Dhima K, Eleftherohorinos I (2005). Allelopathic potential of Bermuda grass and Johnson grass and their interference with Cotton and Corn. Allelopathy J. 97: 303-313.

Makkar HPS, Becker K, Sporer F, Wink M (1997). Studies on nutritive potential and toxic constituents of different provenances of *J. curcas*. J. Agric. Food Chem. 45: 3152–3157.

Maldonado JAC, Osornio JJ, Barragan TA, Anaya AL (2001). The use of allelopathic legume cover and mulch spocies for weed control in cropping system. Agron. J. 93: 27-36.

Mehmood S, Orhan I, Ahsan Z, Aslan S, Gulfraz M (2008). Fatty acid composition of seed oil of different Sorghum bicolor varieties. Food Chem. 109: 855-859.

Molish H (1937). Der Einfluss einer Pflanze auf die andere-Allelopathie, Gustav, Fischer, Verlag, Jena.

Muniru KTA, Hassanali TA, Hooper AM (2003). Isoflavanones from the allelopathic aqueous root exudate of *Desmodium uncinatum*, Phytochemistry, 64: 265-273.

Naengchomnong W, Thebtaramonth Y, Wiriyachitra P, Okamoto KT, Clardy J (1986). Isolation and Structure Determination of Two Novel Lathyrane from Jatropha curcas. Retrahedron Lett. 27: 5675-5678.

Narwal SS (1999). Allelopathy Update. Basic and Applied Aspects. Sci. Pub. Enfield.

Olofsdotter M, Navarez D, Rebulanan M, Streibig JC (1999). Weed suppressing rice cultivars-Does allelopathy play a role. Weed Res. 39: 441-454.

Putnam AR, Tang CS (1986). The Science of Allelopathy. John Wiley and Sons, New York.

Rice EL (1984). Allelopathy, 2nd ed. Academic Press, Orlando,FL. p. 422 .

Sampietro DA, Vattuone MA, Isla MI (2005). Plant growth inhibitors isolated from sugarcane (*Saccharum officinarum* L.) straw. J. Plant Physiol. 163: 837–846.

Sampietro DA, Vattuone MA (2006) Sugarcane straw and its phytochemicals as growth regulators of weed and crop plants. Plant Growth Regul. 48: 21–27.

Seigler DS (1996). Chemistry and mechanisms of allelopathic interactions. Agron. J. 88: 876–885.

Sun RC, Sun XF (2001). Identification ans quantitation of lipophilic extractives from wheat straw. Ind. Crops. Prod. 14: 51-64.

Wink M, Koschmieder C, Sauerwein M, Sporer F (1997) Phorbol esters of J. curcas Biological activities and potential applications. In Gubitz GM, Mittelbach M & Trabi M (eds). Biofuel and Industrial Products from J. curcas. Graz: Dbv-Verlag Univ. pp. 160–166.

Wu HW, Haig T, Pratley J (2001). Allelochemicals in wheat (*Triticum aestivvum L.*): production and exudation of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one, J. Chem. Ecol. 27: 188-194.

Wu L, Guo X, Harivandi MA (1998). Allelopathic effect of phenolic acids detected in buffalograss (*Buchloe dactyloides*) clippings on growth of annual bluegrass (*Poa annua*) and buffalograss seedlings, Environ. Exp. Bot. 39: 159-167.

Zeng RS, Luo S, Shi YH (2001). Physiological and Biochemical Mechanism of Allelopathy of Secalonic Acid F on Higher Plants. Agron. J. 93: 72-79.

Zhang ZL (1990). Experiments and methods in plant physiology. (In Chinese). 2nd ed. Higher Educ. Press. Beijing.