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Different Vegetables Crops in Response to Allelopathic of Hot Pepper Root Exudates

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Abstract: Laboratory bioassay was carried out to investigate the effect of allelopathy of hot pepper root exudates on tomato, corn (used as fresh), lettuce, radish, cucumber, Chinese cabbage, *Brassica oleracea*, cowpea and hot pepper. Indices such as germination rate, germination index and synthetical effect index were measured and the major allelochemicals of hot pepper root exudates were identified using gas chromatography-mass spectrometry. The results suggested that root exudates from hot pepper had allelopathic potential, which differed depending on the concentration of root exudates and the difference of the receiver vegetables. Through the analysis of synthetical effect index, hot pepper, cowpea, cucumber and corn showed a stimulatory response to hot pepper root exudates under lower concentration, whereas they showed an inhibitory response under higher concentration. Hot pepper root exudates exhibited a positive effect on radish and tomato. However, this effect weakened with increasing concentration. Lettuce, Chinese cabbage and *B. oleracea* showed an inhibitory response under all concentrations. Eight kinds of allelochemicals were identified from the hot pepper root exudates. The main component of the allelochemicals was dibutyl phthalate, which may play an important role in allelopathy.

Key words: Allelopathy · Root exudates · Hot pepper · Allelochemicals · Seedling growth

INTRODUCTION

Hot pepper (*Capsicum frutescens* L.) is an important Solanaceae crop species widely cultivated worldwide. It is used in various foods and is recognized as a higher value-added crop [1]. However, consecutive cultivation for several years on the same land of this hot pepper species can lead to serious problems including declining production and quality [2]. This continuous cropping obstacle of hot pepper may be attributed to allelopathy.

Allelopathy is defined as the chemical interactions occurring between and among plants and microorganisms via the release of biologically active chemical compounds into the environment [3]. Allelopathy, which includes stimulatory and inhibitory effects, has been demonstrated in nature by Muller, Weston and Einhellig. Allelopathy plays an important role in both natural and agro-ecosystems. Especially, allelopathy has potential in integrated weed management. Crop plants have the capability to produce allelochemicals into their surroundings to affect plants in their vicinity through volatilization from their surfaces, leaf leachates, root exudates and decomposition. Root exudation is a common way of releasing allelochemicals, which has been proven via bioassay in many crops, such as cucumber [7,8] and hairy vetch [9,10].

Currently, no study has reported on the allelopathic effect of hot pepper root exudates on different vegetables. The current study provides better understanding on the allelopathy mechanisms of hot pepper and offers a reference for the cultivation of hot pepper in the field.

MATERIALS AND METHODS

Plant Materials: Seeds of hot pepper (*Capsicun annuum* L. cv. Lameichang) and other tested vegetables, including cucumber (*Cucumis sativus* L. cv. Jinyan), lettuce (*Lactuca sativa* L. cv. Xiangyou9), cabbage (*Brassica oleracea var. capitata* L. cv. Niuxin), Chinese cabbage (*Brassica campastris* L. cv. Zhaoshu5), tomato (*Lycopersicon esculentum* L. cv. Hezuo908), radish (*Raphanus sativus* L. var. Hongyou), cowpea (*Vigna unguiculata* L. cv. Chengjiang7) and corn (*Zea mays* L. cv. Chaotian 605), were obtained from the Horticultural Laboratory of Sichuan Agriculture University.

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Preparation of Root Exudates: Seeds hot pepper were surface sterilized with 70% alcohol for 5 min, soaked at 55.8 °C for 30 min, rinsed four times with distilled water and kept for germination on wet filter paper in Petri dishes at 29 °C. After 4 days seeds were planted into plastic pots. A total of 60 thirty-day-old seedlings with uniform sizes were transplanted into glass cups containing 200 ml Hoagland's nutrient solution, which was prepared using deionized water. Root exudates were collected every 3 days and then the nutrient solution was changed. Root exudates were collected five times. The collected liquid was filtrated through a column (diameter 20 mm) containing 100 ml of XAD-4 resin, followed by elution with 200 ml methanol and evaporation on a rotary evaporator (Model RE-2000) at 40°C. The solution, with a total volume of 25 ml, was then refrigerated at-20°C until use.

Bioassay: Twenty milliliter concentrated methanol solution was diluted with sterile distilled water to a volume of 250 ml. Subsequently, 0, 2 and 5 ml of diluted solution of root exudates were obtained and added with distilled water to a volume of 10 ml, configuring into three concentrations of treatment solutions (represented by CK, A and B, respectively).

The treatment solution (10 ml) was added to a filter paper placed in sterilized 9 cm glass Petri dishes containing the tested seeds. Thirty seeds of each vegetable were surface sterilized with 70% alcohol for 5 min, washed thoroughly with distilled water and evenly placed on filter papers each placed in a Petri dish in a dark chamber at 25°C. Number of germinated seeds was recorded daily and the germination rate was counted over a seven-day period. Germination was deemed to occur only after the radicle had protruded beyond the seed coat by at least 1 mm. The length and weight of the radicle and hypocotyl of the seedlings were measured after 10 days.

$$\text{Germination rate} = \text{final germination} / \text{total seeds} \times 100\%$$

The germination index (GI) was calculated according to Wardle *et al.* [11]:

$$GI = \Sigma(Gt/Dt)$$

where Gt is the number of germinated seeds and Dt is the days of the germinated seeds.

The synthetical effects of hot pepper root extracts on tested crops were concluded according to synthetical effect index (SEI), which is the average of every response index (RI). A stimulatory effect was demonstrated if SEI >0, whereas an inhibitory effect was demonstrated if SEI <0. The strength of allelopathy was determined according to the absolute value of SEI. RI was demonstrated using the method of Williamson and Richardson [12]:

$$RI = 1\text{-}C/T\ (T \geq C)\ RI = T\square C\text{-}1\ (T<C)$$

where C and T represent the value of the control and treatment, respectively.

Identification of Root Exudates: Concentrated methanol solution (5 ml) was transferred to a XAD-4 adsorption resin column (4 cm in length, 2 cm in diameter) with 200 ml 80% ether + 20% ethyl acetate elution to allow the natural evaporation of methanol. The eluate was concentrated under vacuum to dryness and then dissolved in 5 ml of methanol. The main component was used in the identification of the root exudates through gas chromatography-mass spectrometry (GC-MS, GC Agilent 6890, MS HP5973) analysis.

Statistical Analysis: Germination and seedling growth bioassays were conducted in a complete randomized design with three replications. One-way analysis of variance was carried out using the SPSS software package Version 17.

RESULTS

Effect of Hot Pepper Root Exudates on Seed Germination Rate: The allelopathy potential of hot pepper root exudates was evaluated using nine vegetable seeds as the tested plants at two different concentrations of exudates collected during the period of seedling development in the culture system (Fig. 1). The results showed that the effect of hot pepper root exudates on the germination rate of *B. oleracea*, lettuce, radish, cucumber, cowpea and hot pepper exhibited a significant difference compared with the control. The germination rate of hot pepper was increased significantly under treatment A, but decreased under treatment B. In addition, radish and cowpea improved under all concentrations, whereas the other vegetables were inhibited with increasing concentration.

Effect of Hot Pepper Root Exudates on Seed Germination Index: Data indicated that the allelopathy of root exudates on seed germination index was similar with the germination rate (Fig. 2). It also indicated that the germination index of all vegetables, except for tomato,

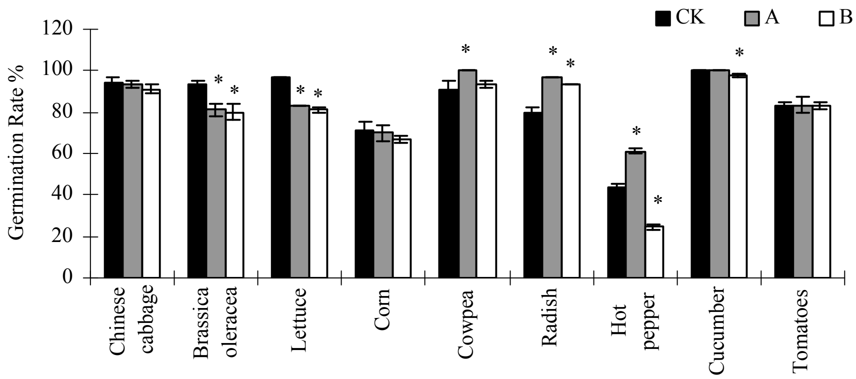

Fig. 1: Effect of hot pepper root exudates on the seed germination rate of the receiver crop. Mean pairs followed by the asterisks are significantly different (P < 0.05) via Duncan's test. CK, control; A, lower concentration of root exudates from hot pepper; B, higher concentration of root exudates from hot pepper.

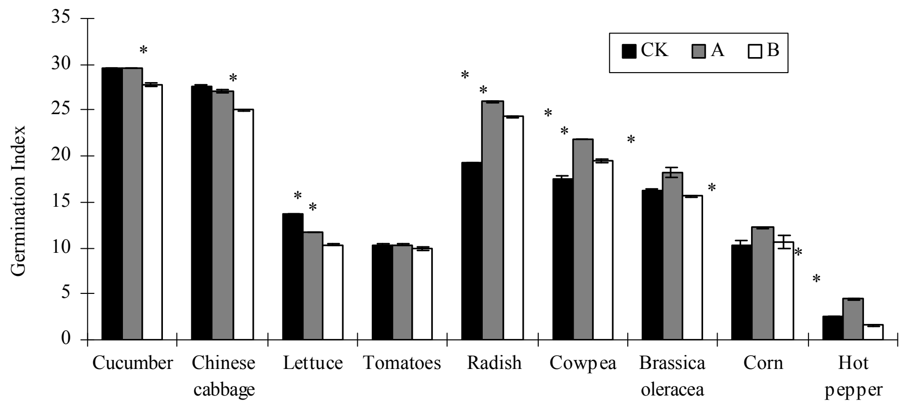

Fig. 2: Effect of hot pepper root exudates on the seed germination index of the receiver crop.

showed a significant difference compared with the control under concentration A or B. The index of radish and cowpea improved significantly under the two concentrations. In addition, hot pepper was promoted via treatment A, but was inhibited via treatment B. However, the germination index of corn and *B. oleracea* seemed different with its germination rate.

Mean pairs followed by asterisks are significantly different ($P < 0.05$) via Duncan's test. CK, control; A, lower concentration of root exudates from hot pepper; B, higher concentration of root exudates from hot pepper.

Effect of Hot Pepper Root Exudates on Seedling Growth: The allelopathic potential of the collected root exudates was tested through its effect on the seeding growth of the receiver crop (Table 1). The seedling growth of tomato and radish was improved but decreased with increasing concentration. The growth of other seedlings, such as cowpea, cucumber, corn and hot pepper was promoted by treatment A, but was inhibited by treatment B. The seedling growth of Chinese cabbage, *B. oleracea* and lettuce was reduced under all concentrations compared with the control.

Synthetical Effects of Root Extracts from Hot Pepper on the Tested Vegetables: The stimulatory or inhibitory synthetical effects of hot pepper root extracts on the tested vegetables were evaluated through SEI (Table 2). Hot pepper, radish and cowpea showed greater allelopathic responses under treatment A, whereas hot pepper, radish and *B. oleracea* were more sensitive to allelopathy compared with the other vegetables under treatment B. This result suggested that allelopathic potential varied depending on the concentration of root exudates and the difference of the tested vegetables. Among the tested vegetables, hot pepper showed the greatest stimulatory or inhibitory response under treatment A or B, respectively. Radish showed the largest

Table 1: Allelopathic effect of hot pepper root exudates on seedling growth of the receiver crop

Receiver crops	Shoot height (cm)	Root length (cm)	Top fresh mass (mg plant^{-1})	Root fresh mass (mg plant^{-1})
CK	3.60±0.16	7.43±0.62	165±26.84	90±0.83
Cucumber A	4.05±0.14*	7.58±0.82	187±7.32	88±3.31
B	4.06±0.12*	7.23±0.63	165±4.96	73±0.79*
CK	8.79±0.49	3.83±0.78	620±45.02	74±0.61
Cowpea A	9.98±0.44	6.10±0.54*	785±20.06*	89±0.55*
B	7.65±0.45	2.34±0.42	516±46.16	61±4.13*
CK	2.30±0.19	5.09±0.55	13±0.58	5±0.24
Tomato A	3.51±0.17*	7.61±0.32*	24±3.69*	15±0.37*
B	2.40±0.16	5.58±0.79	21±0.37*	5±0.33
Hot pepper CK	0.40±0.06	0.70±0.28	15±0.87	4±0.18
A	0.70±0.04*	1.68±0.09*	21±0.83*	10±1.45*
B	0.34±0.06	0.53±0.11	15±0.75	3±0.22
Chinese cabbage CK	3.09±0.12	3.16±0.44	37±0.56	8±0.15
A	2.49±0.14	2.63±0.5	34±2.83	9±0.3
B	2.38±0.13*	1.84±0.32*	31±0.6*	8±0.12
Brassica oleracea CK	1.89±0.13	2.90±0.47	30±0.58	5±0.2
A	1.79±0.17	2.06±0.38	24±1.52	4±0.1
B	1.48±0.14	1.78±0.20*	22±0.59*	4±0.3
CK	4.48±0.14	3.55±0.49	107±3.82	25±0.58
Radish A	6.06±0.13*	4.31±0.18	129±15.67	35±0.81*
B	5.61±0.18*	3.94±0.51	117±3.45	32±0.55*
CK	3.19±0.08	3.86±0.12	22±1.36	6±0.23
Lettuce A	2.85±0.08	2.98±0.17*	17±0.67	4±0.32*
B	2.16±0.11*	2.24±0.14*	15±0.55*	3±0.12*
CK	6.28±0.14	10.08±0.78	475±6.09	530±21.95
Corn A	6.6±0.26	13.91±0.95*	536±7.05*	688±1.88*
B	5.8±0.18	9.96±0.48	445±1.59*	518±2.09

Note: Mean pairs followed by asterisks are significantly different ($P < 0.05$) via Duncan's test. CK, control; A, lower concentration of root exudates from hot pepper; B, higher concentration of root exudates from hot pepper.

Table 2: Synthetical effects of root extracts from hot pepper on different vegetables

Treatments		RI_{GR}	RI_{GI}	RI_{GRO}	SEI
Cucumber	A	0	0	0.058	0.019
	B	-0.02	-0.06	-0.028	-0.036
Cowpea	A	0.09	0.20	0.218	0.169
	B	0.02	0.10	-0.215	-0.032
Tomato	A	0	0.00	0.460	0.153
	B	0	-0.04	0.163	0.041
Hot pepper	A	0.29	0.44	0.479	0.403
	B	-0.44	-0.38	-0.151	-0.324
Chinese cabbage	A	-0.01	-0.02	-0.081	-0.037
	B	-0.04	-0.09	-0.204	-0.111
Brassica oleracea	A	-0.13	0.11	-0.100	-0.04
	B	-0.14	-0.04	-0.218	-0.133
Radish	A	0.17	0.26	0.223	0.218
	B	0.14	0.21	0.152	0.167
Lettuce	A	-0.14	-0.14	-0.122	-0.134
	B	-0.03	-0.12	-0.227	-0.126
Corn	A	-0.02	0.15	0.168	0.099
	B	-0.05	0.03	-0.043	-0.021

Note: RI_{GR} represents the average RI of germination rate; RI_{GI} represents the average RI of germination rate; RI_{GRO} represents the average RI of seeding growth; SEI is the synthetical effect index. A, lower concentration of root exudates from hot pepper; B, higher concentration of root exudates from hot pepper.

Table 3: Allelochemicals in hot pepper root exudates

Allelochemicals	Percentage/%	Similar degree	Time of apex
Dibutyl phthalate	40.2	90	23.88
1,2-benzenedicarboxylic acid, butyl cyclohexy ester	16.8	88	21.79
1,2-benzenedicarboxylic acid, butyl 2-methylpropyl ester	12.73	80	24.58
1,2-benzenedicarboxylic acid,bis(2-methylpropyl) ester	10.2	91	22.15
Diphenylamine	9.27	85	18.26
4, 4'-(1-methylethylethylidene)bis-phenol	4.71	87	31.12
Benzene naphthylamine	3.56	93	27.28
1,2-benzenedicarboxylic acid	2.53	91	17.31

stimulatory effect, but the positive effect weakened with concentration. Lettuce showed the largest inhibitory effect under all concentrations.

Analysis of Allelochemicals in Hot Pepper Root Exudates: The standard pattern of GC-MS showed eight kinds of high-level substances in the root exudates. Dibutyl phthalate was found to have the highest content, which was 40.2% (Table 3).

DISCUSSION

Several plant species have been reported to exhibit allelopathic activity on the growth of other plant species [13-15]. However, previous studies have focused on crops and weeds and the results were different according to plant species. Thi *et al.* [8]observed that the extracts of cucumber plants inhibited the growth of cress and lettuce; however, radish exhibited no allelopathic response to hairy vetch[10]. The present study is the first to prove the reciprocal allelopathic responses in hot pepper and nine other vegetables. This study revealed that hot pepper roots exhibit allelopathic potential and can produce allelochemicals, which are released into the environment via decomposition of plant residues in sufficient quantities to affect neighboring or successional plants.

Allelochemicals are low molecular weight compounds excreted from plants during secondary metabolism processes[16]. Hot pepper root exudates were identified and the results confirmed that dibutyl phthalate was the main allelochemical, which may play a major role on other vegetables. However, the specific role of each component in the allelochemicals still needs further study.

The current study suggested that the effect of hot pepper root exudates on different vegetables was dependent on the concentration of the extracts. In addition, hot pepper root exudates can inhibit the growth of lettuce and Chinese cabbage, whether with high concentrations or low concentrations. Moreover, hot pepper root exudates improved the growth of radish and tomato, but this positive effect weakened with increasing concentration. In other words, most of the tested vegetables exhibited allelopathic inhibition with higher concentrations of hot pepper root exudates. Similar findings were also proven by numerous studies in other plants [17,18].

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