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# Potential allelopathic effects of allelochemicals in aqueous extracts of leaves and root exudates of *Capsicum annuum* on vegetable crops

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## ABSTRACT

Phytotoxic effects of aqueous extracts of leaf (control (CK), 0.5, 1, 2, 4 and 8 %, w/v) and root exudates (CK, 10d, 20d, 30d, 40d, 50d) of *Capsicum annuum* were studied under laboratory condition on seed germination and seedling growth of 6 test vegetable crops [Hot pepper (*Capsicum annuum* L.), tomato (*Lycopersicon esculentum* Mill.), cucumber (*Cucumis sativus* L.), radish (*Raphanus sativus* L.), pumpkin (*Cucurbita moschata* Duch.) and lettuce (*Lactuca sativa* L.)]. Both aqueous extracts and root exudates inhibited the seed germination and seedling growth of lettuce, and their inhibitory effects increased with increasing concentration and increase in the duration of cultivation time. In aqueous extracts of leaves and root exudates of *C. annum*, 28-compounds (alkanes, phthalate ester, phenols anilines and carboxylic acid) were isolated and identified by GC and GC-MS. The bioactivity of 15-suspected allelochemicals were studied on seed germination and seedling growth of *Lactuca sativa*. Two main inhibitory substances, N-phenyl-2-naphthylamine, phthalic acid, were found as potential allelochemicals of *Capsicum annuum*, with inhibitory effects on seed germination and seedling growth of lettuce. Both N-phenyl-2-naphthylamine, phthalic acid, showed separate and synergetic potential allelopathic effects.

**Keywords**: Allelochemicals, allelopathy, aqueous extracts, *Capsicum annuum*, phytotoxic effects, potential allelochemicals, root exudates, test plant.

## INTRODUCTION

Allelopathy is defined as the beneficial or harmful influence of chemical substances mainly derived from the secondary metabolism of plants, microorganisms and fungi that influence the growth and development of nearby plants or microorganisms, and interfere with ecosystem structure and dynamic (2,6). Most allelochemicals are low molecular weight compounds which are produced in different plant tissues including leaves, stems, roots, flowers, seeds, barks, and buds (16) and released into the environment by root exudation, natural vaporization, leaching and decomposition (8) depending on their physico-chemical properties and on the specific site of accumulation and/or production. There are different concentration of allelochemicals in each department (10), and the quantity and emission pathway vary within species. Most plants exhibit harmful or beneficial allelopathic effects on seed germination, seedling growth and development of

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other plants in the vicinity by accumulating allelochemicals into the soil and other organisms (15,22,27). The effects of aqueous extracts on seed germination and plant growth is used to evaluate the allelopathic interactions among plants (30). Allelochemicals can also indirectly influence neighboring species by modifying biotic and abiotic interactions, such us nutrient uptake and bioavailability (24) or disturb microorganism population in the soil (9). The high concentrations of allelochemicals may negatively influence other plants, low concentration may be stimulating, a phenomenon known as hormesis (11).

*Capsicum annuum L.,* (Solanaceae family), is major vegetable and cultivated worldwide. Its decomposed leaves inhibited the germination and growth of weed species in laboratory bioassays and 5-phenolic compounds were identified (18), hence, we hypothesized that *C. annuum* has potential allelopathic effects. Thus we assessed its phytotoxic effects and identified the potential allelopathic chemical substances and evaluated their phytotoxic effects on seed germination and seedling growth of test crops under controlled laboratory conditions. Seed germination and seedling growth are primary bioassay tools for determining the phytotoxic activity (12).

This laboratory study aimed (i). to determine the allelopathic potential of aqueous extracts of leaves and root exudates of *C. annuum* on seed germination and early seedling growth (root length and shoot length) of 6-test plants and (ii). identify the main compounds and their concentrations in aqueous extracts of leaves and root exudates.

## MATERIALS AND METHODS

Continuous cropping obstacles among vegetable crops can result in soil degradation, diseases and insects, low quality and so on. Hot pepper (*Capsicum annuum* L.), tomato (*Lycopersicon esculentum* Mill.), cucumber (*Cucumis sativus* L.), radish (*Raphanus sativus* L.*)*, pumpkin (*Cucurbita moschata* Duch.) and lettuce (*Lactuca sativa* L.) are common vegetables in everyday life, so they were selected as test plant according to allelopathic effects. Their seeds were obtained from Daqing Agriculture Academy of Science, Heilongjiang, China. The study was conducted during June-August in 2013. The experimental treatments consisted of 3-factors: (i). 6-Test crops (hot pepper, tomato, cucumber, radish, pumpkin and lettuce), (ii). 6-Concentrations (0, 0.5, 1, 2, 4, 8%) of hot pepper aqueous leaf extracts and root exudates and (iii) root exudated collection time (10, 20, 30, 40, 50 days). All experimental treatments were arranged in completely randomized design with four replications.

All reagents were purchased from Kermel Chemical Reagent Co., Ltd. (Tianjin, China). The individual stock solutions of exogenous suspected allelochemicals were prepared by dissolving each compound in methanol. The standard solutions were prepared by diluting stock solution with distilled water (TTL-6, Beijing, China) to required concs.

### I. Preparation of aqueous extracts of leaves and collection of root exudates

**Aqueous leaf extracts:** The hot pepper leaves were collected from the median part of 60-day-old lettuce, firstly, the lettuce were dried at 70℃ for 30 min, then dried at 80℃ for 4 hours. The dried leaves were cut into 1-cm pieces and ground in blender (5). Leaf dry powder (50 g) was soaked and stirred in 250 mL distilled water for 24 h at 25℃. The stock solution (20%, w/v) was prepared by filtering the supernatant through two layers of Whatman No.1 filter paper. For bioassay, stock solution was diluted to test concentrations (0.5, 1, 2, 4, 8%) with distilled water.

**Root Exudates:** We used the continuous root exudates trapping system (CRETS) to trap root exudates compounds in a column (15 mm dia.) containing 200 mm XAD-4 resin (Sigma, USA) (23). For bioassay, the root exudates solution were collected at different periods (10 d, 20 d, 30 d, 40 d, 50 d). Blank tests were done using culture solution without plants and distilled water to eliminate the effects of external factors, respectively. The different aqueous extracts from leaves and root exudates of *C. annuum* were kept at 4℃ to determine their potential allelopathic effects and extraction of organic compounds.

**II. Bioassay**

The potential allelopathic effects of aqueous extracts from leaves and root exudate of *C. annuum* were tested for 6-test plants (hot pepper, tomato, cucumber, radish, pumpkin and lettuce). Uniform seeds of test plants were sterilized with 5% solution of sodium hypochlorite for 5 min and then rinsed five times with distilled water. Thirty seeds of each test species were placed per Petri dish (90 mm dia) as per treatments on filter paper treated with aqueous leaf extract and root culture solution. Aqueous solution (5 mL) from leaves at six concentrations (CK,0.5,1,2,4 and 8 %) and collected root culture solution at different periods (CK, 10 d, 20 d, 30 d, 40 d, 50 d) were added to each Petri dish, respectively, and Hoagland's nutrient solution without plants was used as the control. The Petri dishes were kept in a growth chamber [temperature: 20℃, relative humidity: 70%, 16 h light/8 h dark] for 7 days. Germination (%) was determined at 24 h intervals for 7 days. Thereafter, the root length (mm) and shoot length (mm) were recorded. All experimental treatments were arranged in a completely randomized design with four replications.

Sterilized hot pepper seeds were placed in Petri dish (90 mm dia.) between two sheets of moist Whatman No.1 filter paper. 15 days old seedlings were transferred in a growth chamber (GRP-9080, Shanghai, China), then 20 uniform seedlings were transplanted into 2 L half Hoagland's nutrients solution. The hot pepper plants were grown in greenhouse for 50 days without supplementary illumination at day/night temperatures: 20-25/12-16℃ and Relative humidity: 70% - 80%. The hydroponic Hoagland's nutrient solution was prepared using distilled water and changed every 10 days. The pH of nutrient solution and electrical conductivity were maintained at 6.0 and at 0.5$\mu S \cdot cm^{-1}$, respectively, by daily measurement to ensure that no substantial changes occurred in the nutrient solution throughout the experiment. Plant sampling and sample preparation were done (4).

**III. Osmotic potential**

This bioassay was conducted to evaluate the sensitivity of germination and early seedling growth of six test plants to osmotic potential of aqueous extracts prepared from leaves and root exudates. The osmotic potential of each treatment was determined using a thermocouple psychrometer (Wescor, Logan, Utah). According to the osmotic potential of extracts (-2, 1.5, 1.0, 0.5 and 0 MPa, respectively), five polyethylene glycol (PEG) 6000 molecular weight solutions were prepared at different concentrations by dissolving different PEG in distilled water. Different PEG solution (distilled water as control) was added to the Petri dishes containing 30-test plant seeds. The temperature and light conditions during seed incubation and seedling growth were similar to previous bioassays. Distilled water was added to the Petri dish to maintain constant osmotic potential by weighing daily, and germination rate, germination potential, root length and shoot length were measured and calculated as described in bioassays within 7 days.

**IV. Extraction and quantification of potential allelopathic substances**

The aqueous solution from leaves extracts at 20% (the treatment method is same as above) were filtered through Whatman No. 1 filter paper, and then filtered again through a 0.45 µm membrane (Xinya Co., Shanghai, China). Root culture solution in the last 10-days was directly extracted. The solution was separated into four fractions (neutral compounds, phenols, alkaloids, and organic acids) by adjusting the solution pH: 3 or PH:12 by either HCl or NaOH. The extraction procedure was modified from Shui *et al*. (21). After separation, each fraction was evaporated to dryness at 40 ℃ by rotary evaporator (R-210, Buchi) after dehydrating with $CaSO_4$, then brought to volume of 1mL by ethylacetate for analysis.

The GC-MS experiments were performed using a GC 2010 gas chromatography coupled to a QP mass spectrometer (Shimadzu, Japan) operating in full scan in the range m/z 50-500 and selected ion monitoring (SIM) mode for analytes. The column used was a Rxi-5ms (5% diphenyl/95% dimethylpolysiloxane) fused silica capillary column (30 m long×0.25 mm i.d×0.25 µm film thickness). The initial temperature of column was set at 50℃ and held for 1 min, then increased to 250 ℃ at a rate of 15 $℃\cdot min^{-1}$ and held for 2 min, and finally increased to 270 ℃ at a rate of 20 $℃\cdot min^{-1}$ and held for 6min.The injector temperature was set at 250 ℃. The MS operating conditions were as follows: Helium (99.999%) was employed as carrier gas and its rate was adjusted to 3 mL•min-1 in constant flow mode. The ion source and transfer line temperatures were set at 200 ℃ and 280 ℃, respectively. The electron energy (70 eV) was operated in the EI mode.

**Response Index :** To evaluated the effects of treatments, the response index (*RI*) was calculated as under (28):

$$\text{If } T \geq C\text{; then } RI = 1 - C/T\text{; If } T < C\text{; then } RI = T/C - 1$$

Where, T: Germination rate or growth response of test plant treated with undiluted aqueous extracts from leaf and root exudates and C: Germination rate or growth response of test species in control (Hoagland's nutrient culture solution without plants).

Positive *RI* value: Stimulatory effects of hot pepper-derived extract on germin-ation or seedling growth, Negative *RI* value : Inhibitory effects of hot pepper-derived extract on germination or seedling growth.

Subordinative function value was applied to evaluate synthetically allelopathic potential, it avoided inaccuracy of single index, so the method can provide more scientific and reliable reference. Subordinative function value were calculated as under:

$$Zy = \frac{Xy - Xtmin}{Xtmax - Xtmin}$$

If there was negative correlation between the index and allelopathic potential, calculations were done as under:

$$Zy = 1 - \frac{Xy - Xtmin}{Xtmax - Xtmin}$$

Where, $Zy$ : Subordinative function value of j index for i plant, $Xy$ : Determination value of j index for i plant and $Xtmax$ , $Xtmin$ : Maximum value and minimum value of every index, respectively.

**V. Statistical analysis:** Data were analyzed using a one-way ANOVA test in SAS12.0. Mean values were taken from measurements of four replications and standard errors of the mean calculated. All measurements were subjected to Duncan's multiple range test to discriminate significance ($p < 0.05$). Excel and Origin was used to draw figures.

## RESULTS AND DISCUSSION

**Seed germination:** The aqueous extracts from leaves and root exudates of *C. annuum* at 0.5% concentration slightly stimulated the seed germination of lettuce than control (Table 1). Potential allelopathic effects of leaf extracts and root exudates gradually increased with increasing treatment concentration and extended cultivation time of *C. annum*. Aqueous leaf extract inhibited the seed germination at 1% concentration and inhibitory effects gradually increased with increasing treatment concentration i.e. concentration dependent. Root exudate showed potential allelopathic effects during the 20 days' cultivation, and there was significant difference with the extension of cultivation period. The determined inhibitions of leaf extracts were more pronounced than that of root exudates for the germination rate. The seed germination of lettuce were inhibited by 57.8% and 40.3% at 8% treatment concentration and 50 days' cultivation for leaf extracts and root exudates, respectively. The results showed that potential allelochemicals in leaf extracts and root exudates could have a harmless or harmful effect on seed germination of the lettuce. Therefore, potential allelopathic effects of *C. annuum* includes not only in aqueous leaf extracts but also in the root exudates.

**Seedling growth :** Leaf extracts showed stimulative effects for root length of lettuce seedlings at the concentration of 0.5% (Fig.3a). However, it showed significant inhibitory effect at 1%, and the inhibitory effect was relatively enhanced with increasing extract concentration (Fig. 3a). It can be concluded that the low extracts concentrations could stimulate the root elongation of lettuce, however, high concentration had harmful effects on root elongation. Root exudates solution in different period showed the same change regularity as leaf extract. The results obtained from root elongation tests also support the findings of germination tests (Fig. 3b). Some allelochemicals can stimulate the growth of plants while having neither harmful nor beneficial effects on the seed germination (13).

Table 1. The potential allelopathic effects of leaves extract and root exudates of *C. annuum* on test plants

| Test plant | GR | | GP | | RL | | SL | | Sum | |
|---|---|---|---|---|---|---|---|---|---|---|
| | LE | RE | LE | RE | LE | RE | LE | RE | LE | RE |
| *C. annuum* | 0.518 | 0.493 | 0.468 | 0.431 | 0.322 | 0.304 | 0.334 | 0.298 | 1.642 | 1.526 |
| *L. esculentum* | 0.948 | 0.858 | 0.849 | 0.789 | 0.824 | 0.719 | 0.754 | 0.647 | 3.375 | 3.013 |
| C. *sativus* | 0.529 | 0.487 | 0.618 | 0.527 | 0.467 | 0.424 | 0.438 | 0.348 | 2.052 | 1.786 |
| *R. sativus* | 0.927 | 0.841 | 0.719 | 0.647 | 0.759 | 0.657 | 0.710 | 0.627 | 3.115 | 2.772 |
| *C. moschata* | 1.187 | 0.912 | 0.621 | 0.587 | 0.757 | 0.678 | 0.803 | 0.712 | 3.368 | 2.889 |
| *L. sativa* | 0.417 | 0.323 | 0.331 | 0.297 | 0.304 | 0.229 | 0.328 | 0.308 | 1.380 | 1.157 |

GR: Germination rate; GP: Germination potential; RL: Root length; SL: Shoot length; LE: Leaf extract; RE: Root exudate

Table 2. Effects of different osmotic potentials of polyethylene glycol solutions on germination rate (GR) of test plant

| Osmotic | Germination Rate GR (%) | | | | | |
|---|---|---|---|---|---|---|
| potential (MPa) | *C. annuum* | *L. esculentum* | *C. sativus* | *R. sativus* | *C. moschata* | *L. sativa* |
| -2 | 80.4a | 68.7a | 75.7a | 86.1a | 94.2a | 61.7a |
| -1.5 | 81.0a | 67.8a | 75.1a | 84.1a | 93.4a | 61.1a |
| -1.0 | 78.9a | 67.2a | 74.5a | 84.2a | 93.2a | 60.5a |
| -0.5 | 81.2a | 67.8a | 73.8a | 85.8a | 92.8a | 60.8a |
| 0 | 79.9a | 68.5a | 73.2a | 85.2a | 92.2a | 62.2a |

*Data represent mean value of each parameter; the same lowercase letters indicate no significant difference at 5% after applying Duncan's multiple range test.

Table 3. Effects of different osmotic potentials of polyethylene glycol solutions on root length (RL) and shoot length (SL) of test plant

| Osmotic potential | *C. annuum* | | *L. esculentum* | | *C. sativus* | | *R. sativus* | | *C. moschata* | | *L. sativa* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (MPa) | RL | SL | RL | SL | RL | SL | RL | SL | RL | SL | RL | SL |
| -2 | 40.5a | 40.7a | 40.7a | 42.4a | 46.2a | 52.7a | 57.2a | 62.5a | 51.6a | 71.2a | 29.7a | 31.5a |
| -1.5 | 42.2a | 39.9a | 39.9a | 43.1a | 45.4a | 53.9a | 55.9a | 61.9a | 50.7a | 70.1a | 31.2a | 29.8a |
| -1.0 | 40.9a | 40.1a | 38.9a | 41.9a | 45.8a | 53.1a | 56.1a | 63.4a | 49.9a | 72.4a | 30.7a | 30.5a |
| -0.5 | 40.1a | 41.8a | 39.6a | 42.7a | 44.3a | 52.4a | 55.4a | 64.0a | 51.2a | 71.8a | 31.9a | 31.4a |
| 0 | 41.8a | 40.4a | 40.6a | 41.4a | 44.9a | 51.8a | 55.7a | 62.9a | 50.7a | 72.6a | 32.0a | 30.2a |

*Data represent mean value of each parameter; the same lowercase letters indicate no significant difference at 5% after applying Duncan's multiple range test.

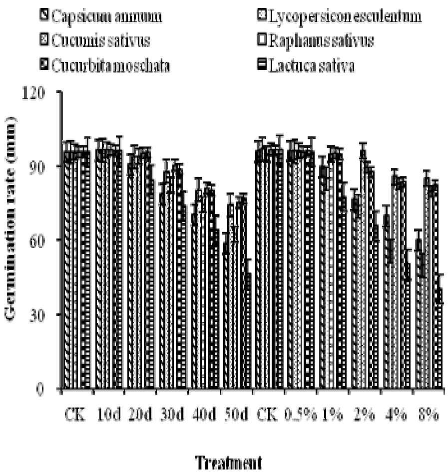


Figure 1. Effects of leaf extracts and root exudates on germination rate of 6-test plants.

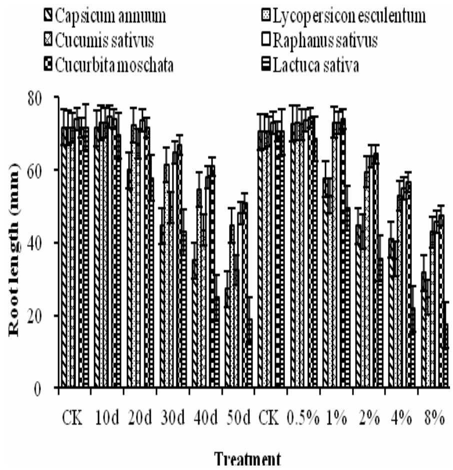


Figure 2. Effects of leaf extracts and root exudates on root length of 6-test plants.

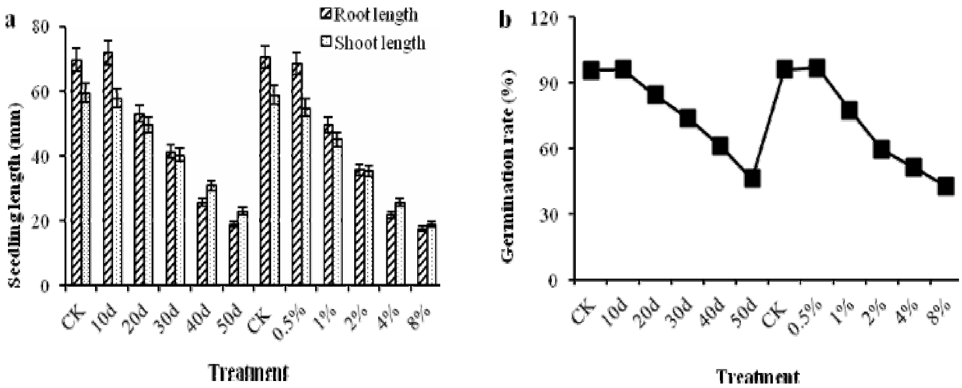


Figure 3. Effect of leaf extract and root exudates on the germination rate and seedling length in the lettuce.

Inhibitory effects of leaf extracts and root exudates on shoot length and root length increased with increasing treatment concentration and the extension of cultivation time, respectively. The results showed that potential allelochemicals in the leaf extracts and root exudates could have both beneficial and harmful effects on seedling growth of the lettuce. It was determined that leaf extracts and root exudates exhibited variable allelopathic potential on seed germination and seedling growth of the lettuce (1). Seedling growth was a more sensitive indicator for the allelopathic potential of leaf extracts and root exudate of *C. annuum* compared to seed germination.

Phytotoxic effects might be ascribed to differential phytotoxic strength provided by allelochemicals variability of *C. annuum*. The phytochemicals having phytotoxic properties in leaf extracts and root exudates are the dependence of species-specific. Therefore, *C. annuum* may be considered as an phytotoxic species, presenting both harmful and harmless effect to seed germination and seedling growth of the lettuce.

**Osmatic potential:** The GR of six test plant seeds were not affected by different osmotic potential of PEG solutions (Table 2). Similarly, neither RL nor SL of six test plant were significantly influenced by different concentrations PEG solution (Table 3).

Aqueous extracts from leaves and root exudates of *C. annum* significantly inhibited the root length and germination rate of all test species and inhibitory effects increased with increasing concentration of treatmenst and the extension of cultivation time (Fig. 1 and Fig. 2). RI (Response Index) of root exudates for germination rate and root length ranged between -0.583~＋0.002 and -0.737~＋0.017 and that of leaf extract was between -0.602 ~＋0.014 and -0.753~＋0.074 , respectively, for different test plants. The greater the absolute value of RI, the stronger was the allelopathic effect and the RI were highest for germination rate and root length in the lettuce, i.e. the inhibition in germination rate and seedling growth in *Lactuca sativa* was maximum than all test plants. However, potential allelopathic effect of plant is reflected by individual indicator that has one-sidedness and the limitation, subordinative function value can reflect the allelopathic potential scientifically and systematically, so comprehensive subordinative function value were applied to measure the potential allelopathic effects, the smaller the SFV of test plant, the stronger was the allelopathic response. Lettuce was the most appropriate test plant, owing to its lowest SFV for leaf extract and root exudates of *C. annuum* (Table 1). Besides, lettuce is also a good test plant with quick germination and high sensitivity, hence, most used in researches (14,17,25,29). Thus, lettuce was considered as test plant in the further allelopathic research.

**Identification of potential allelochemicals**

Twenty-eight compounds (similarity ⩾80%) including alkanes, phthalate ester, phenolsanilinesand carboxylic acid were isolated and identified from the aqueous leaf extracts and root exudates by GC-FID and GC-MS (Table 4). Fifteen organic compounds were [DMP, DEP, DIOP, DIBS, DIBA, MP, 2,3-DMP, 2,6-DTBP, 4,4'-(1-M)BP, 1-NA, DPA DIBP, DBP, N-P-2-NA and PA] suspected allelochemicals (3,19). To study their potential allelopathic effects, these exogenous substances were applied for lettuce seed bioassay. Besides the allelopathic potential of aqueous extracts from leaves and root exudates of hot pepper was evaluated at 0, 2, 4, 6, 8, 10 $\mu g \cdot mL^{-1}$ concentrations on seed germination and seedling growth of lettuce. The DMP, DEP, DIOP, DIBS, DIBA, MP, 2,3-DMP, 2,6-DTBP, 4,4'-(1-M) BP, 1-NA, DPA, DIBP and DBP did not influence the seed germination and seedling growth of lettuce < $10 \mu g \cdot mL^{-1}$. The N-phenyl-2-naphthylamine and phthalic acid at < 2 $\mu g \cdot mL^{-1}$ did not influence the germination rate, germination potential, root length and shoot length of lettuce, however, > 4 $\mu g \cdot mL^{-1}$ they inhibited the seed germination and seedling growth and inhibitory effects increased with increasing concentrations. Mixed treatment of N-phenyl-2-naphthylamine and phthalic acid (3:2) were applied to lettuce culture solution as per the detected true concentrations (Table 4), N-P-2-NA and PA showed the allelopathic inhibitory effects, therefore, N-phenyl-2- naphthylamine and phthalic acid may work as potential allelopathic substances in the following study according to allelopathy definition and research method of allelopathic potential.

Table 4. The components of root exudates and leaf extract of *C. annum*

| No. | Class | Formula | Compound | Abbreviation | Concentration ($\mu g \cdot mL^{-1}$) | |
|---|---|---|---|---|---|---|
| | | | | | RE | LE |
| 1 | Alkanes | $C_{11}H_{24}$ | Undecane | – | nq | nq |
| 2 | | $C_{12}H_{26}$ | Dodecane | – | nq | nq |
| 3 | | $C_{13}H_{28}$ | Tridecane | – | nq | nq |
| 4 | | $C_{14}H_{30}$ | Tetradecane | – | nq | nq |
| 5 | | $C_{15}H_{32}$ | Pentadecane | – | nq | nq |
| 6 | | $C_{16}H_{34}$ | Hexadecane | – | nq | nq |
| 7 | | $C_{17}H_{36}$ | Heptadecane | – | nq | nq |
| 8 | | $C_{18}H_{38}$ | Octadecane | – | nq | nq |
| 9 | | $C_{19}H_{40}$ | Nonadecane | – | nq | nq |
| 10 | | $C_{20}H_{42}$ | Eicosane | – | nq | nq |
| 11 | | $C_{21}H_{44}$ | Heneicosane | – | nq | nq |
| 12 | | $C_{24}H_{50}$ | Tetracosane | – | nq | nq |
| 13 | | $C_{13}H_{12}$ | Diphenylmethane | – | 4.98 | nd |
| <u>14</u> | Esters | $C_{10}H_{10}O_4$ | Dimethyl phthalate | DMP | 5.12 | 4.45 |
| <u>15</u> | | $C_{12}H_{14}O_4$ | Diethyl phthalate | DEP | 5.04 | 3.64 |
| <u>16</u> | | $C_{16}H_{22}O_4$ | Diisobutyl phthalate | DIBP | 7.27 | 5.57 |
| <u>17</u> | | $C_{16}H_{22}O_4$ | Dibutyl phthalate | DBP | 6.43 | 5.28 |
| <u>18</u> | | $C_{24}H_{38}O_4$ | Diisooctyl phthalate | DIOS | 4.71 | 3.77 |
| <u>19</u> | | $C_{12}H_{22}O_4$ | Diisobutyl succinate | DIBS | 3.96 | 3.68 |
| <u>20</u> | | $C_{14}H_{26}O_4$ | Diisobutyl adipate | DIBA | 4.48 | 3.56 |
| <u>21</u> | | $C_{17}H_{34}O_2$ | Diisobutyl phthalate | MP | 3.99 | 3.46 |
| <u>22</u> | Phenols | $C_8H_{10}O$ | 2,3-Dimethylphenol | 2,3-DMP | 8.96 | 6.89 |
| <u>23</u> | | $C_{14}H_{22}O$ | 2,6-Di-tert-butylphenol | 2,6-DTBP | 8.59 | 6.74 |
| <u>24</u> | | $C_{15}H_{16}O_2$ | 4,4'-(1-Methylethyliden) bisphenol | 4,4'-(1-M)BP | 7.44 | 5.84 |
| <u>25</u> | Anilines | $C_{10}H_9N$ | 1-Naphthylamine | 1-NA | 4.67 | 3.48 |
| <u>26</u> | | $C_{12}H_{11}N$ | Diphenylamine | DPA | 6.18 | 4.52 |
| <u>27</u> | | $C_{16}H_{13}N$ | N-phenyl-2-naphthalene amine | N-P-2-NA | 7.60 | 6.58 |
| <u>28</u> | Carboxylic acid | $C_8H_6O_4$ | Phthalic acid | PA | 5.17 | 4.58 |

*–: No abbreviation; RE: Root exudates ; LE: Leaf extracts; nq: Not quantitative; nd: Not detected.
–: (Underline) : Suspected allelochemicals.

The regression analysis of two potential allelochemicals (N-phenyl-2-naphthylamine and phthalic acid) on germination rate, germination potential, root length and shoot length was studied. When lettuce seeds were treated with different concentrations (0,2,4,6,8,10 $\mu g \cdot mL^{-1}$) of N-phenyl-2-naphthylamine, phthalic acid and combination of N-phenyl-2-naphthylamine and phthalic acid, there was no influence on germination (%), germination potential, root length than control. While shoot length of lettuce was decreased progressively with increasing treatment concentrations. At the 10 $\mu g \cdot mL^{-1}$, N-phenyl-2-naphthylamine caused 49.6% ,61.9%, 68.5% and 64.6% inhibition and phthalic acid caused 42.8%, 52.4%, 59% and 53.6% inhibition in germination rate (%),

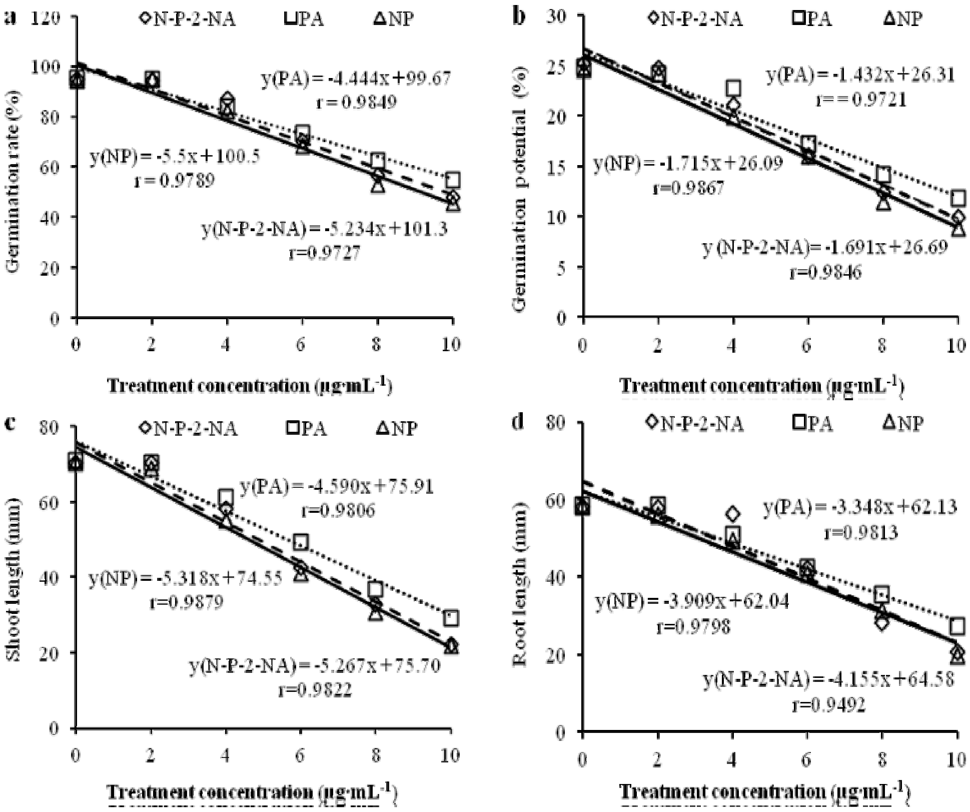


Figure 4. Regression analysis of two exogenous potential allelochemicals on seed germination and seedling length of the lettuce.

germination potential, root length and shoot length of lettuce, respectively, than control (Fig. 4). Thus in exogenous treatment, the concentration of potential allelochemicals significantly influenced the germination rate, germination potential, root length and shoot length of lettuce. The mixed treatment of N-phenyl-2-naphthylamine and phthalic acid was more inhibitory than individual compounds. At the same concentration the N-phenyl-2-naphthylamine was more phytotoxic than phthalic acid and mixed treatment of N-P-2-NA and PA showed significant synergistic effects on germination rate, germination potential, root length and shoot length.

Generally it is supposed that the inhibitory effects of plant extracts on seed germination or seedling growth is caused by phytotoxins, however, the extracts may also exert negative osmotic effects on the test species (26). In this study, differences in growth parameters could not be attributed to osmotic potential effect, because tests with different PEG concentrations did not influence the germination and growth of test plant. Potential allelochemicals of aqueous extracts from leaves and root exudates were studied in hydroponic culture solution, and species-selective inhibitory effect and additive or synergistic effect of allelochemicals were also studied (7,20). However, there is difference between the hydroponics system and field conditions, hence, long-term field studies and further phytotoxic assays are needed to support this proposition from the practical standpoint, and more studies on the allelopathic mechanism of *C. annuum* in test plant under field condition is also needed in future studies.

## CONCLUSIONS

Aqueous extracts of leaves and root exudates of *C. annuum* inhibited the seed germination and seedling growth of lettuce possibly by releasing or secreting the water-soluble organic compounds. The phytotoxic effects on seed germination and seedling growth increased with increasing concentrations of aqueous extracts of leaves and root exudates of *C. annuum*. Twenty-eight compounds were isolated and identified from the aqueous extracts of leaves and root exudates. The N-phenyl-2-naphthylamine and phthalic acid may be considered as two potential allelochemicals of root exudates and leaf extracts in *C. annuum*. The N-phenyl-2-naphthylamine was more phytotoxic than phthalic acid in the hydroponic system. The mixed treatment of N-phenyl-2-naphthylamine and phthalic acid showed the synergistic effects.

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## REFERENCES

1. Bouhaouel, I., Gfeller, A., Fauconnier, M.L., Rezgui, S., Amara, H. S. and Jardin, P. (2014). Allelopathic and autotoxicity effects of barley (*Hordeum vulgare* L. ssp. vulgare) root exudates. *BioControl*. 10.1007/s10526-014 -9634-3.
2. Amini, S., Azizi, M., Joharchi, M. R., Shafei, M. N., Moradinezhad, F. and Fujii, Y. (2014). Determination of allelopathic potential in some medicinal and wild plant species of Iran by dish pack method. *Theoretical and Experimental Plant Physiology*. 26:189-199.
3. Callaway, R.M. and Aschehoug, E.T. (2000). Invasive plants versus their new and old neighbors: A mechanism for exotic invasion. *Science* **290**: 521-523.
4. Chung, I.M., Seigler, D., Miller, D.A. and Kyung, S.H. (2000). Autotoxic compounds fr*om fresh* alfalfa leaf extracts: identification and biological activity. *Journal of Chemical Ecology* **26**: 315-327.
5. Dapkevicius, A., Venskutonis, R., Van Beek, R. and Linseen, J.P.H. (1998). Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. *Journal of Science Food Agriculture* **77**: 140-146.
6. De Albuquerque, M.B., Dos Santos, R.C., Lima, L.M., Melo Filho, P.A., Nogueira, R.J.M.C., Da Camara, C.A.G. and Ramos, A.R. (2011). Allelopathy, an alternative tool to improve cropping systems. A review. *Agronomy. For Sustainable Development* **31**: 379-395.
7. Einhellig, F.A., Schon, M.K. and Rasmussed, J.A. (1982). Synergistic effects of four cinnamic acids compounds on grain sorghum. *Journal of Plant Growth Regulation* **1**: 251-258.
8. Fernandez, C., Monnier, Y., Ormeńo, E., Baldy, V., Greff, S., Pasqualini, V., Mévy, J. P. and Bousquet-Mélou, A. (2009). Variations in allelochemical composition of leachates of different organs and maturity stages of *Pinus halepensis*. *Journal of Chemical Ecology* **35**: 970-979.

9. Fernandez, C., Santonja, M., Gros, R., Monnier, Y., Chomel, M., Baldy, V. and Bousquet-Mélou, A. (2013). Allelochemicals of *Pinus halepensis* as drivers of biodiversity in Mediterranean open mosaic habitats during the colonization stage of secondary succession. *Journal of Chemical Ecology* **39**: 298-311.
10. Gatti, A.B., Ferreira, A.G., Arduin M., Andrade G. and Perez, S.C. (2010). Allelopathic effects of aqueous extracts of *Artistolochia esperanzae* O. Kuntze on development of *Sesamum indicum* L. seedlings. *Acta Botanica Brasilica*. **24**: 454-461.
11. Hadacek, F., Bachmann, G., Engelmeier, D. and Chobot, V. (2011). Hormesis and a chemical raison d'être for secondary plant metabolites. *Dose-Response* **9**: 79-116.
12. Inderjit and Dakshini, K.M.M. (1995). On laboratory bioassays in allelopathy. *Botanical Rev*iew **61**: 28-44.
13. Macias, F.A., Oliva, R.M., Varela, M., Torres, A. and Molinillo, M.G. (1999). Allelochemicals from sunflower leaves cv. Peredovick. *Phytochemistry* **52**: 613-621.
14. Nishihara, E., Parvez, M.M., Araya, H., Fujii, Y. (2004). Germination growth response of different plant species to allelochemical L-3,4-dihydroxyphenylalanine (L-DOPA). *Plant Growth Regulation* **42**: 181-189.
15. Noguchi, H.K. and Ino, T. (2005). Possible involvement of momilactone B in rice allelopathy. *Journal of Plant Physiology* **162**: 718-721.
16. Parvez, S., Parvez, M., Fujii, Y. and Gemma, H. (2004). Differential allelopathic expression of bark and seed of *Tamarindus indica* L. *Plant Growth Regulation* **42**: 245-252.
17. Pennacchio, M., Jefferson, L.V. and Havens, K. (2005). *Arabidopsis thalian*a: A new test species for phytotoxic bioassays. *Journal of Chemical Ecology* **131**: 1877-1885.
18. Reigosa, M.J., Souto, X.C. and Gonźlez, L. (1999). Effects of phenolic compounds on the germination of six weeds species. *Plant Growth Regulation* **28**: 83-88.
19. Rice, E. L. (1984). *Allelopathy*. II Ed. Academic Press, Orlando, Florida. pp. 231-233.
20. Scognamiglio, M., D'Abrosca, B., Esposito, A., Pacifico, S., Monaco, P. and Fiorentino, A. (2013). Plant growth inhibitors: Allelopathic role or phytotoxic effects? Focus on mediterranean biomes. *Phytochemistry Reviews* **12**: 803-830.
21. Shui, J.F., An, Y., Ma, Y.Q. and Ichizen, N. (2010). Allelopathic potential of switchgrass (*Panicum virgatum* L.) on perennial ryegrass (*Lolium perenne* L.) and alfalfa (*Medicago sativa* L.). *Environment Management* **46**: 590-598.
22. Singh, H.P., Batish, D.R. and Kohli, R.K. (2003). Allelopathic interactions and allelochemicals: New possibilities for sustainable weed management. *Critical Reviews in Plant Sci*ences **22**: 239-311.
23. Tang, C.S. and Young, C.C. (1982). Collection and identification of allelopathic components from the undisturbed root system of bigalta limpograss (*Hemarthria altissinia*). *Plant Physiology* **69**: 155-160.
24. Tharayil, N. ( 2009) . To survive or to slay. *Plant Signal Behav*ior **4**: 580-583.
25. Thi, H.L., Toshiaki, T., Kiyotake, S., Chin, D.V. and Kato-Noguchi, H. (2008). Allelopathy and the allelopathic activity of a phenylpropanol from cucumber plants. *Plant Growth Regulation* **56**: 1-5.
26. Wardle, D.A., Nicholson, K.S. and Ahmad, M. (1992). Comparison of osmotic and allelopathic effects of grass leaf extracts on grass seed germination and radical elongation. *Plant and Soil* **140:** 315-319.
27. Weir, T.L., Park, S.W. and Vivanco, J.M. (2004). Biochemical and physiological mechanisms mediated by allelochemicals. *Current Opinion in Plant Biology* **7**: 472-479.
28. Williamsom, G.B. and Richardson, D. (1988). Biossays for allelopathy: Measuring treatment response with independent controls. *Journal of Chemical Ecology* **14**: 181-188.
29. Zeng, H.Y., Alan, A.R. and Saxena, P.K. (2009). Evaluation of *in-vitro* shoots of *Artemisia judaica* for allelopathic potential. *Acta Physiologiae Plantarum* **31**: 1237-1248.
30. Zhu, H. *and M*allik, A.U (1994). Interactions between *Kalmia* and black spruce: Isolation *and identification of alle*lopathic compounds. *Journal of Chemical Ecology* **20**: 407-421.