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Allelopathic effects of root exudates from wheat, oat and soybean on seed germination and growth of cucumber

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ABSTRACT

We examined the allelopathic effects of root exudates from wheat, oat and soybean on germination and growth of cucumber seedlings. We used a special device to collect the root exudates and studied their allelopathic effects. wheat, oat and soybean root exudates had different allelopathic effects on seed germination of cucumber. Wheat root exudates at higher concentrations promoted the seed germination, but low concentrations were inhibitory. A similar pattern was observed with exudates from soybean, but the opposite trend was observed with oat roots exudates. These three types of root exudates also had variable effects on the seedling growth of cucumber. The integrated allelopathy index (*RI*) showed that most root exudates promoted the seedling growth than other treatments and control. The wheat root exudates of 50% concentration were most stimulatory to growth (*RI* = 3.333), followed by 100% concentration (*RI* = 2.488). The higher concentrations of wheat root exudates stimulated the seed germination and seedling growth of cucumber. Contrarily, oat root exudates at 100% concentration inhibited the seedling growth of cucumber.

Key words: Allelopathic effects, cucumber, germination, oat, root exudates, seedlings, soybean, wheat

INTRODUCTION

Allelopathy refers to the biotic interactions in which a plant or microorganism releases the bioactive metabolites into the environment, which favourably or unfavourably affects the growth of other plants or organisms in the vicinity (16). Inter-plant allelopathy is widely recognized, e.g., the formation and succession of vegetation, seed germination and decay in agro-ecosystems. Examples of allelopathic relationships in agriculture include inter-cropping, inter-planting, mixed-cropping, crop-rotation etc., where root exudates may play significant role in the crop-crop interactions(8,15,25). Root exudates contain numerous allelopathic substances, which influences the growth and root activities of other plants (6,10). Therefore, to optimize agricultural production, we should understand the allelopathic interactions in inter-cropping, inter-planting, mixed-cropping and crop rotations.

In China, serious problems arise particularly during the continuous cultivation of cucumber, perhaps due to the changes in soil microorganisms spp. and population

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(10,15,19). Our recent research has focussed on inter-cropping, or inter-planting and crop rotations in cucumber crop and aims (i). to restore the diversity in the microbial community and (i). to improve the functioning of rhizosphere ecosystem. Because the sustainability of agriculture depends on such ecological measures (13,14,21).

In this study, we examined the allelopathic effects of root exudates from wheat, oat and soybean on the germination and growth of cucumber. So that these results can be used to plan most appropriate inter-cropping and inter-planting systems and crop rotations for sustainable cucumber cultivation, through control of problems arising from the continuous cropping.

MATERIALS AND METHODS

The certified seeds of cucumber (*Cucumis sativus* L.) cv. 'Tianjin Green 3'; wheat (*Triticum aestivum* L.) cv. 'Kefeng 6'; soybean (*Glycine max*) cv. 'North 86-4' and oat (*Avena sativa* L.) cv. 'Baiyan II' were purchased from various sources.

Collection and processing of root exudates

Seeds of wheat, oat and soybean were sown in wooden boxes. When seedlings were 15-20 cm tall, they were removed from the soil. Rhizosphere soil was removed with running water and then roots were washed thrice in distilled water and in deionized water (13). To collect root exudates, we selected 3200 wheat seedlings (45 days old), 4800 oat seedlings (45 days old) and 1200 soybean seedlings (56 days old). To collect the root exudates, the roots of these seedlings were immersed in deionized water (1.5-2.0 cm deep) in the collection device (Photographs 1 and 2) for 72 h (23-28 ^{o}C and 2-5 million Lux light). The quantity of root exudates collected for 72 h (May 16-19, 2008) per pot was 3162 ml (wheat), 2639 ml (oat) and 4161 ml (soybean) and filtered through 0.22 µm membrane. The volume of each extract was made to 5 L with deionized water, so that total volume of all these exudates was same. Although the concentration of root exudates was different, but it was considered as 100% concentrations of wheat, soybean, oats exudates and was stored at -20°C until use (21). The root exudates from wheat, soybean and oat were each diluted with deionized water to prepare 12.5,25,50 and 100 % concentrations.

Table 1. The Symbols used for different concentrations (%) of root exudates

Crop	100	50	25	12.5
Wheat	A1	A2	A3	A4
Soybean	B1	B2	B3	B4
Oat	C1	C2	C3	C4

Lab Bioassay

The experimental treatments consisted of two factors: (i). Test Crops : 3 (Wheat, oat, soybean) and (ii). Root exudates concentrations: 4 (12.5,25,50,100 %). The treatments were replicated thrice in completely Randomised Design. Two layers of filter paper were placed in Petri dishes (10-cm dia) and 6 mL root exudates solution was added per Petri

Photograph 1

Photograph 2

Photographs 1 and 2. The Photographs showing the device used to collect the root exudates.

dish as per treatments. Then, 30 pre-soaked cucumber seeds were placed evenly in each dish and the dishes were placed in light incubator at 30°C for seeds to germinate (2). Deionized water was used as control (CK).

Pot study

The experimental treatments were same as per Lab bioassay, but here studies were done in Pots. We placed soil (sieved through 6 mm sieve to remove the plant residues etc.) from a continuously cultivated cucumber crop field into pots (300 g per 10 × 15 cm pot). Root exudates were applied at 300 ml/pot and deionized water was used as control (CK). Two cucumber seeds were sown per pot. These experiments contained 13 treatments in total (3 crop plants × 4 concentrations of exudates + 1 control), and carried out 3 times replications, We used 20 pots for each treatment, making a total of 780 pots.

In lab.bioassay, we recorded the cucumber seeds germination at 36 h after incubation, germination rate at 48 h, radicle length at 72 hours and fresh and dry weight at 192h (26). In pot culture growth indices of cucumber seedlings, were determined at 30-d after sowing. Values obtained were mean of three separate determinations.

Root activity was determined using the TTC method (26); photosynthetic net rate by using L1-6400 Portable Photosynthetic System (LI-COR, Lincoln, USA). The activity indices were calculated as under:

Germination rate (%) × seedling dry weight (g) (11,17).

To determine the effect of root exudates on cucumber seedlings, the sensitivity index (*RI*) as per Williamson (22) was calculated as under:

$$RI = 1 - C/T \ (T \geq C)$$

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

Where, C : control and T : treatment value. *RI* values > 0 Growth promotion and values < 0 : Growth inhibition. SE is the cumulative allelopathic index of cucumber seedling growth and was calculated as under: SE= Allelopathic index of root length + Allelopathic index of root fresh weight + Allelopathic index of plant height + Allelopathic index of stem thickness + Allelopathic index of number of leaves + Allelopathic index of plant fresh weight (17,22).

Statistical analysis

Each sample was measured thrice and the average was calculated. We used Excel and SAS 6.0 software to analyze data. Significant differences are indicated by lower-case letters ($P < 0.05$).

RESULTS

Lab Bioassay

Seeds germination

The root exudates from three crops had different effects on cucumber seed germination index (Table 2). Wheat root exudates at higher concentrations promoted the germination, but lower concentrations were inhibitory. Root exudates from soybean had also similar effects. Whereas the root exudates from oat stimulated the germination at lower concentrations and inhibited at high concentration. Compared with controls, the highest germination rate of seeds was observed with 50% soybean root exudates (B2; *RI*=0.131) and followed by 50% wheat root exudates (A2; *RI* = 0.088). That is the highest germination potential in cucumber seeds was with 50% wheat root exudates, followed by seeds treated with 50% soybean root exudates, then treated with 100% wheat root exudates. All other exudates solutions inhibited the germination potential and the most drastic inhibition was found in seeds treated with 12.5% soybean root exudates (B4; –0.158). The seeds lowest germination rate was observed with 12.5% wheat root exudates (A4; *RI* = –0.060) followed by 50% oat root exudates (C2; *RI* = –0.024).

Wheat root exudates at high concentrations increased the germination vigour index, whereas, lower concentrations reduced it (Table 2). A similar pattern was observed in the soybean exudates treatments. All concentrations of oat root exudates solutions reduced the germination vigour index. The maximum stimulation in germination vogour index was seen with 50% wheat root exudates (A2; *RI* = 0.114) followed by 50% soybean root exudates (B2; *RI* = 0.082). The strongest inhibition was observed with 12.5% wheat root exudates (A4; *RI* = –0.227).

Seedlings growth

Most root exudates treatments increased the seedlings' fresh weight (Table 2). The higher concentrations of wheat root exudates increased the cucumber fresh weight, while low concentrations, decreased it. Soybean root exudates at higher concentrations increased the fresh weight of cucumber seedlings but oats root exudates did not show any effects.

Table 2. Effects of root exudates on cucumber seed germination and shoot growth (*RI*)

Treatment	Germination rate (%)	Germination potential (%)	Vigour index	Shoot fresh weight (g)	Radicle length (cm)	Lateral roots
CK	0 f	0 d	0 d	0 i	0 f	less
A1	0.076 ± 0.099 c	0.034 ± 0.111 c	0.044 ± 0.252 c	0.138 ± 0.123 b	0.216 ± 0.0065 a	more
A2	0.088 ± 0.129 b	0.180 ± 0.113 a	0.114 ± 0.138 a	0.147 ± 0.186 a	0.141 ± 0.0045 b	more
A3	0.040 ± 0.086 e	-0.155 ± 0.171 h	-0.495 ± 0.355i	0.063 ± 0.108 e	-0.046 ± 0.0055g	same
A4	-0.060 ± 0.148 h	-0.130 ± 0.154 f	-0.227 ± 0.349 h	-0.080 ± 0.239 j	0.027 ± 0.0045 d	same
B1	0.053 ± 0.143 d	-0.129 ± 0.114 f	0.045 ± 0.239 c	0.014 ± 0.296 h	0.099 ± 0.0075 c	less
B2	0.131 ± 0.049 a	0.142 ± 0.061 b	0.082 ± 0.142 b	0.112 ± 0.234 d	0.120 ± 0.0060 bc	more
B3	0.059 ± 0.088 d	-0.129 ± 0.043 f	0.069 ± 0.060 bc	0.041 ± 0.327 fg	0.100 ± 0.0121 c	more
B4	-0.001 ± 0.144 f	-0.158 ± 0.233 h	-0.064 ± 0.153 e	0.052 ± 0.270 f	0.139 ± 0.0060 b	same
C1	-0.020 ± 0.094 g	-0.084 ± 0.165 e	-0.057 ± 0.326 e	0.133 ± 0.118 c	0.041 ± 0.0485 e	less
C2	-0.024 ± 0.136 g	-0.130 ± 0.187 f	-0.131 ± 0.211 g	0.127 ± 0.052 c	0.100 ± 0.0061 c	same
C3	0.040 ± 0.086 e	-0.060 ± 0.168 e	-0.299 ± 0.648 g	-0.001 ± 0.084 i	0.210 ± 0.0160 a	less
C4	0.001 ± 0.095 f	-0.154 ± 0.155 h	-0.071 ± 0.201 ef	0.036 ± 0.092 g	0.116 ± 0.0075 c	less

Note: Different letters following values indicate significant difference ($P < 0.05$).

Table 3. Effects of root exudates of different crops on cucumber seedlings growth (*RI*)

Treat-ment	Root length	Root fresh weight	Plant height	Stem thickness	Number of leaves	Plant fresh weight	SE
CK	0f	0j	0de	0ef	0e	0g	0g
A1	0.310 ± 0.004a	0.576 ± 0.026b	0.381 ± 0.005a	0.212 ± 0.007a	0.322 ± 0.040a	0.337 ± 0.017a	2.488 ± 0.025b
A2	0.304 ± 0.008a	0.615 ± 0.008a	0.432 ± 0.111a	0.178 ± 0.006ab	0.256 ± 0.029b	0.325 ± 0.007a	3.333 ± 0.021a
A3	0.262 ± 0.004b	0.257 ± 0.009g	0.195 ± 0.010bc	0.196 ± 0.010a	0.257 ± 0.127b	0.257 ± 0.013b	1.425 ± 0.125d
A4	0.244 ± 0.022c	0.198 ± 0.010h	0.198 ± 0.010bc	0.118 ± 0.006bc	0.257 ± 0.009b	0.215 ± 0.010cd	0.967 ± 0.169e
B1	-0.207 ± 0.005j	0.291 ± 0.017f	0.221 ± 0.017b	-0.234 ± 0.007gh	0.164 ± 0.011c	0.215 ± 0.013cd	2.085 ± 0.065c
B2	-0.048 ± 0.006g	0.363 ± 0.010e	0.074 ± 0.057cde	0.074 ± 0.007cd	0e	0.123 ± 0.011e	0.867 ± 0.075e
B3	-0.131 ± 0.004i	0.294 ± 0.018f	0.127 ± 0.046bcd	-0.197 ± 0.013g	0e	0.200 ± 0.014d	0.378 ± 0.017f
B4	0.178 ± 0.006e	0.254 ± 0.008g	-0.021 ± 0.003de	0.199 ± 0.006a	0.130 ± 0.014d	0.071 ± 0.004f	0.459 ± 0.473f
C1	-0.074 ± 0.005h	0.064 ± 0.011i	-0.034 ± 0.039de	-0.044 ± 0.140f	0e	0.065 ± 0.009f	-0.083 ± 0.114g
C2	-0.012 ± 0.004f	0.458 ± 0.008c	-0.243 ± 0.300f	-0.276 ± 0.005h	0.256 ± 0.014b	0.224 ± 0.006c	1.944 ± 0.060c
C3	0.171 ± 0.006e	0.435 ± 0.010d	-0.065 ± 0.008e	0.025 ± 0.007de	0e	0.323 ± 0.012a	1.064 ± 0.050e
C4	0.230 ± 0.004d	0.298 ± 0.012f	0.242 ± 0.007b	0.180 ± 0.006ab	0e	0.055 ± 0.007f	0.333 ± 0.018f

Note: Different letters following values indicate significant difference ($P < 0.05$).

Most of the treatments of root exudates increased the radicle length over the controls. Only the 25% wheat exudates (A3) were inhibitory. The 100% wheat exudates strongly promoted the radicle length and lateral roots in cucumber seedlings. Growth of cucumber roots was promoted with 25% oat root exudates, but their higher concentrations did not have any significant effects. Cucumber seedlings treated with wheat and soybean root exudates had more lateral roots and roots were vigorous in appearance, while those treated with oat root exudates had fewer and slender lateral roots.

Pot Study

Seedlings growth

The shoot growth was promoted by high concentrations of root exudates from wheat and inhibited by low concentrations (Table 3). Plants treated with root exudates from soybean showed a similar trend, while the opposite trend was observed with oat root exudates treatments. The largest growth promotion was observed in 50% wheat root exudates treatment (A2; *RI* = 0.432), followed by 100% wheat root exudates treatment (A1; *RI* = 0.381) and 12.5% oat root exudates (C4; *RI* = 0.242).

Plants treated with root exudates from wheat had larger stem diameter, whether the exudates were applied at high or low concentrations (Table 3). The largest stem diameters were observed in A1, A2, and A3 treatments. There was no influence of soybean root exudates treatments on stem diameter. While the oat root exudates at high concentrations decreased the stem diameter and but low concentrations increased it. All treatments (except B2, B3, C1, C3 and C40 increased the leaf numbers in cucumber seedling than control. Wheat root exudates stimulated the leaf numbers and were maximum in A1 treatment (A1; *RI* = 0.322). All root exudates treatments increased the shoot fresh weight (Table 3). Higher concentrations of wheat root exudates had maximum promotory effects, while, there were no consistent patterns for soybean and oat root exudates treatments.

Roots growth

The different root exudates had varying effects on root length of cucumber seedlings (Table 3). Compared with controls, seedlings treated with wheat root exudates had longer roots and the greatest effect was observed in A1 and A2 treatments. While three highest concentrations of soybean root exudates resulted in shorter roots, but the lowest concentration increased the root length. A similar pattern was observed with oat root exudates treatments.

All root exudates treatments stimulated the root fresh weight (Table 3). The most significant effect was in A2 treatment (A2; *RI* = 0.615), followed by A1 (*RI* = 0.576).

Integrated effect index (SE)

Most of the root exudates promoted the growth of cucumber seedlings (Table 3). Only the C1 treatment inhibited the growth. Wheat root exudates at 50% concentration [A2 (RI: 3.333)], most significantly stimulated the growth followed by A1 (RI : 2.488). The high concentrations of wheat root exudates had stimulatory allelopathic effects on cucumber. Thus wheat is suitable for crop rotation or intercropping with cucumber and might be used for soil remediation in continuous cucumber cropping.

Physiology of cucumber seedlings

Root activity

The root exudates had different effects on the root activities of cucumber (Fig. 1). The root activities of cucumber were higher in treatments A1, A2, A3, B1, B2, B3, C2 and C3 than control. Lower root activities were observed in A4 and C1 treatments. The highest root activities were in treatments A1 and A2, which had allelopathic effect indices of 0.510 and 0.512, respectively. These results suggest that high concentrations of wheat root exudates promoted the activities and growth of cucumber roots. Soybean root exudates had similar effects, while high concentrations of oat root exudates inhibited the root activity, but lower concentrations were stimulatory, although the magnitude of stimulation was smaller than wheat root exudates.

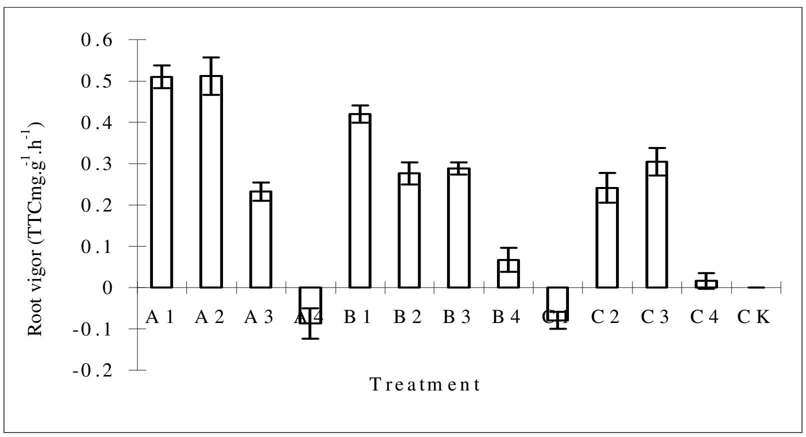

Figure 1. Effects of root exudates on root vigour of cucumber seedlings (*RI*).

Photosynthesis rate

Higher concentrations of wheat root exudates increased the photosynthesis rate in cucumber seedlings (Fig. 2). At lower concentrations, wheat root exudates were inhibitory (A3) or slightly stimulatory (A4). The root exudates from soybean promoted the photosynthesis rate to greater (B3; *RI* = 0.263) or lesser extents (B4). There was no consistent effect of oat root exudates.

DISCUSSION

Root exudates are one of the main pathways for release of allelopathic materials into the environment, they contain various allelopathic substances, whose types and concentrations vary with plant species and its environment (3,5,23). In our study, we found that higher concentrations of wheat root exudates, promoted the cucumber seed germination, germination potential, root activity and root growth. Our results were partly

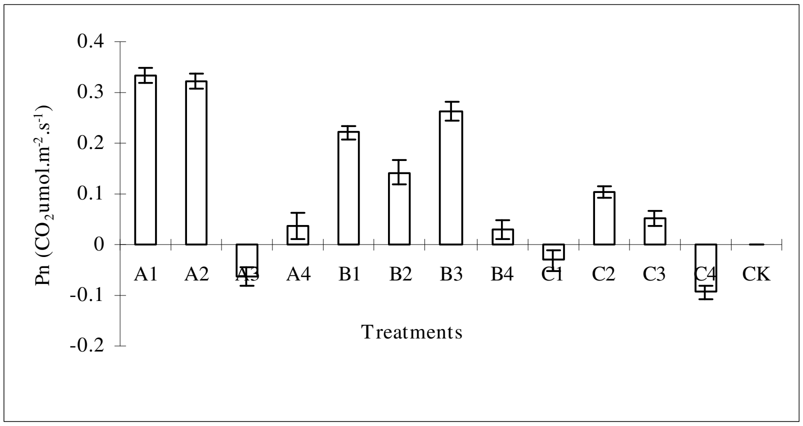

Figure 2. Effects of root exudates on photosynthesis rate of cucumber seedlings (*RI*).

consistent with Yu *et al.* (24). The differences may be owing to varying concentrations or different methods of collecting the exudates. Wheat root exudates and stubble extract inhibits the weeds depending on the wheat varieties. Thus our above results may also be variable due to the test wheat varieties used.

The higher concentrations of wheat root exudates have obviously a catalytic role on cucumber seed germination, this may be due to presence of allelochemicals in wheat root exudates. These allelochemicals promoted the cucumber seed germination at certain concentrations. In wheat root exudates, allelochemicals 'm-cresol(3-methyl-Phenol)' was found in highest concentrations (3) The exogenous m-cresol promoted the maize seed germination above 150×10^{-6}mol/L concentration. However, it is not clear if this stimulation was due to m-cresol or other allelochemicals.

Soybean root exudates increased the cucumber germination and seedling fresh weight, but reduced the germination potential. These exudates also delayed the sprouting of cucumber seeds and increased the lateral growth of cucumber roots. In general, plants treated with soybean root exudates showed reduced shoot length. The different effects of soybean root exudates, that is, promotion vs. inhibition effects, should be studied in more detail in future research. Perhaps the high concentrations of soybean root exudates, inhibited the root length of cucumber seedlings and the inhibition was concentration dependent. The enhanced germination of cucumber at higher concentrations of soybean root exudates, shows that these root exudates contains the allelochemicals which at certain concentration may be stimulatory to α-amylase activity.

Allelopathy works through the allelochemicals. These allelochemicals do not act in isolation but they interact with each other and other chemicals and are also influenced by the environmental conditions, soil environments and plant growth (14). The higher concentrations of oat root exudates inhibited the cucumber seed germination, germination potential and radicle length, but significantly promoted the growth of leaves and plant fresh weight. This showed that allelochemicals after entering the soil, can interact with other chemical compounds present in soil, or may have synergistic effects with variety of other substances and allelochemicals. Plant root exudates contain a complex array of substances, their types and quantities depends on the plant species, stage of growth and development and nutritional status (4,24).

Our present research is relatively small, hence, the future study should determine the synergistic or antagonistic effects of allelochemicals present in (i). plant root exudates (1,7), (ii). different plant parts and (iii). different components in plants mixture.

In our this study, root exudates from three crops showed different allelopathic effects on cucumber seedlings. From the comprehensive allelopathic effects index on seedling growth and physiological indicators, the higher concentrations of wheat root exudates had the greatest effect. Perhaps because the wheat root exudates promoted the root activity and net photosynthetic rate of cucumber seedlings.

CONCLUSIONS

Wheat may be suitable crop as a preceding crop in crop rotation or companion crop for inter-cropping or mixed-cropping with cucumber and may stimulate the cucumber growth and control the soil diseases through its root exudates. The root exudates production starts from 45 days old seedlings. Further research is needed to evaluate the allelopathic effects of root exudates collected from the different phenological phases of plants.

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