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Allelopathic effects of garlic root exudates on germination and seedling growth of some crops and to control *Pythium* species

JUDAN YUAN, JUNSHAN QI, BO ZHANG, YUELI ZHANG, KAI FENG, ZHICAI QU[1] and CHANGSONG LI[*]

Institute of Plant Protection, Shandong Academy of Agricultural Sciences
Shandong Province Key Laboratory of Plant Virology, Jinan 250100, China
E. Mail: changsong.li@yahoo.com

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ABSTRACT

We assessed the allelopathic effects of garlic root exudates on 3-crops (lettuce, rape and radish) and 6-pathogens (*Pythium helicoide, P. irregular, P. ultimum*, *P. violae*, *P. heterothal,* and *P. sylvati*). The water culture method was used to collect the root exudates from garlic between 10 to 60 days of plant growth. In bioassays, the effects of root exudates were determined on seed germination and seedling growth of recipient crops and mycelial growth of 6-pathogens. The garlic root exudates exerted the allelopathic effects on 3-test crops and 6-test pathogens and the magnitude of allelopathic effects depended on the garlic plant age and exudates concentration. The root exudates from 10 to 60 days old plants of garlic, inhibited the germination of all test crops and mycelial growth of pathogens. The inhibitory effects of root exudates were highest from the 30- and 40-days old garlic plants. However, the hypocotyl growth of test crops was stimulated. The individual crop and *Pythium* species differed in their growth responses to the garlic root exudates.

Key words: Garlic, inhibitory effect, lettuce, peanut, *Pythium* spp., radish, rape, root exudates

INTRODUCTION

Allelopathy refers to the inhibitory or stimulatory effects of chemicals released by one plant on the growth and development of other organism (3,5,13). Chemicals released or secreted from the plant roots or shoots may directly inhibit or stimulate the germination, growth and development of other plants and microorganisms (8,10,11,12). Garlic (*Allium sativum* L.) is a perennial herb used in Chinese cuisine and China is its leading producer. Garlic is used as preceding crop in crop rotations to utilize the beneficial effects of its root exudates on succeeding crops. For example, there is less attack of stem rot disease in ginger, when planted after garlic. Research has been done on the antibacterial action of garlic bulb, but little is known about the allelopathy and inhibitory effects of root exudates released at different growth stages of garlic.

Lettuce, rape and radish are common vegetable crops in northern China and sown after garlic in crop rotation. So these 3-crops were selected to test allelopathic effect in this

[*]Correspondence Author. [1]College of Life Science, Qufu Normal University, Qufu 273165, China.

study. To provide the theoretical basis to develop the vegetable based cultivation systems and for the disease prevention and management, we studied the effects of garlic root exudates on seed germination and seedling growth of crops and on mycelial growth of peanut and garlic pathogens.

MATERIALS AND METHODS

The seeds of garlic, lettuce, rape, and radish were obtained from the local seeds market. The *Pythium* pathogens of peanut and garlic were isolated from infected peanut and garlic in our laboratory.

Collection of root exudates

Twenty garlic seeds were planted in each plastic foam board and cultured using distilled water in 15-cm Petri dishes. The garlic root exudates were collected at 10, 20, 30, 40 and 60 days after cultivation. After collecting the root exudates, the corresponding volume of distilled water was added to maintain original level. The garlic root exudates were filtered trough sterile filters under sterile conditions and stored at 4°C.

Photograph 1. Method of collecting the root exudates

Bioassays

(i). Seed germination and seedlings growth of test crops

Thirty seeds of each crop were placed in Petri dishes (12 cm dia) containing 10 ml of garlic root exudates and 2-sheets of filter paper. Four concentrations (1, 1: 2, 1: 4, 1:10) were prepared from the collected garlic root exudates. The Petri dishes were placed in dark at 26°C. The crops seeds germination was recorded daily, while, the root and hypocotyl lengths of seedlings were measured at 4-days after sowing (DAS). Distilled water was used as control.

(ii). Mycelial growth of *Pythium*

The root exudates were mixed with sterilized potato dextrose agar (PDA) medium. Three concentrations (1:10, 1:20 and 1:100) prepared from the collected garlic root exudates were used. The Pythium cake was inoculated on PDA medium plate, with untreated PDA medium as control. The Petri dishes were placed in dark at 26°C. The colony diameters were measured after 2 days.

Statistical analysis

All experiments were repeated 4- times. The DPS statistical software was used for variance analysis. Inhibitory rate, germination and allelopathic effects response index (RI) were calculated using Leather's and Williamson's method (7,15).

RESULTS

Germination

The garlic root exudates drastically inhibited the germination of lettuce, rape and radish, however, the stimulation was also observed (Fig. 1A). The garlic root exudates collected at all stages (10 to 60 DAS) inhibited the germination of all test crops than control. The root exudates were most inhibitory to lettuce followed by radish and least harmful to rape. The root exudates collected at 30 days after sowing (DAS) were most inhibitory, while, those collected at 60 DAS were least deleterious. The germination speed of lettuce and radish decreased, than their respective controls and the difference was not statistically significant for rape. The degree of inhibition depended on the plant age of garlic. The maximum inhibition was observed with root exudates from 30-days-old garlic and the lowest inhibition with root exudates from 60-days-old garlic. At the same plant age, the different crops showed variable responses to garlic root exudates. The multi-factorial statistical analyses indicated significant treatment x plant age interactions for lettuce and radish ($P \leq 0.05$), whose germination was 6.67-49.17% and 4.17-20% lower than control. However, there was little effect on rape germination ($P \leq 0.05$) (Fig. 1 B). The plant age of garlic had significant inhibitory effects on the germination of lettuce and radish treated with root exudates ($P \leq 0.05$), the germination speeds were 4.91-71.08% and 7.98-44.25% lower than control, respectively. Contrarily, the garlic root exudates stimulated the germination speed of rape seeds. The germination speed was lowest (28.92% and 55.75%) with root exudates from 30-day-old garlic and highest (95.09% and 92.02%) with root exudates from 60-day-old garlic plants.

As the garlic root exudates were most inhibitory at 30 DAS, hence, further studies were done to determine the effects of various concentrations of root exudates collected at this stage. There was significant interaction between the germination of lettuce and radish ($P \leq 0.05$) and the concentrations of garlic root exudates (Fig 2. A). The inhibitory effects of root exudates were concentration dependent i.e. the inhibitory effects increased with increasing concentration of root exudates. At the highest concentration (pure root

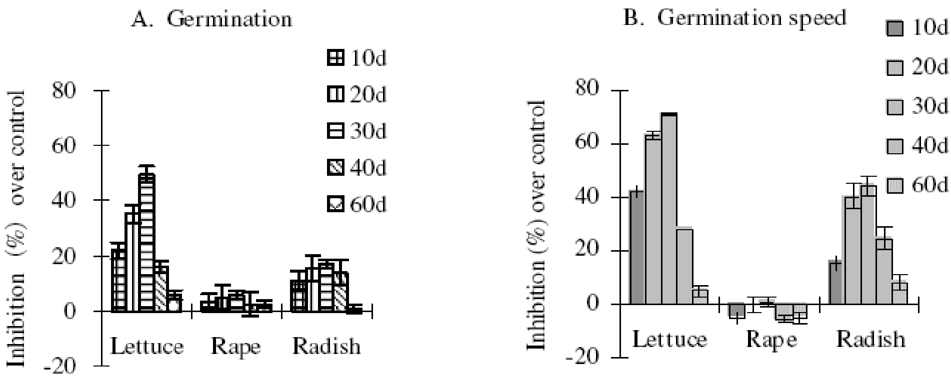

Figure 1.Inhibitory effects of garlic root exudates collected between 10 and 60 days on germination (A) and speed of germination (B) of test crops seeds.

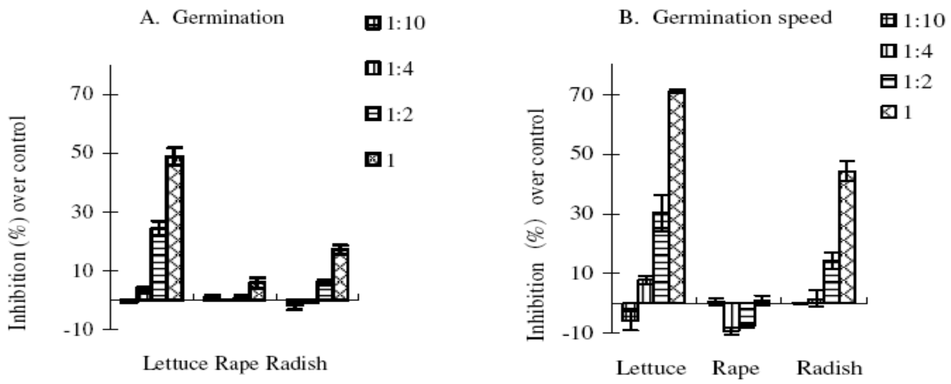

Figure 2. Inhibitory effects of garlic root exudates concentrations collected from 30-days-old plants on germination (A) and speed of germination (B) of test crop seeds.

exudates), the seed germination of lettuce and radish were reduced by 49.17% and 16.67%, respectively. The germination of rape was inhibited only at the highest concentration ($P \leq 0.05$) (Fig. 2 B) The germination speed followed the trend similar to germination. The germination speed of lettuce and radish ($P \leq 0.05$) seeds was inversely related to the concentration of root exudates and the diluted exudates were less potent. At the same concentration, lettuce was most sensitive and rape was most tolerant.

Allelopathic response index (RI): It is an important indicator to determine the allelopathic effects. It was calculated from the germination speeds of control and the treatment. The RI of root exudates for all 3-crops seeds was negative, indicating the inhibitory effects (Fig. 3A). The allelopathic effects on lettuce and radish were strong, but they were slight on rape. The RI of garlic root exudates collected at various stages followed the order (Most Inhibitory to least inhibitory) 30 > 20 >10 > 40 > 60 DAS. As the garlic root exudates were most inhibitory at 30 DAS, hence, further studies were done to determine the effects of various concentrations of root exudates collected at this stage. At lower concentration (1:10), the root exudates from 30-day-old garlic stimulated the germination of Lettuce and rape, but higher concentrations were inhibitory to all test crops

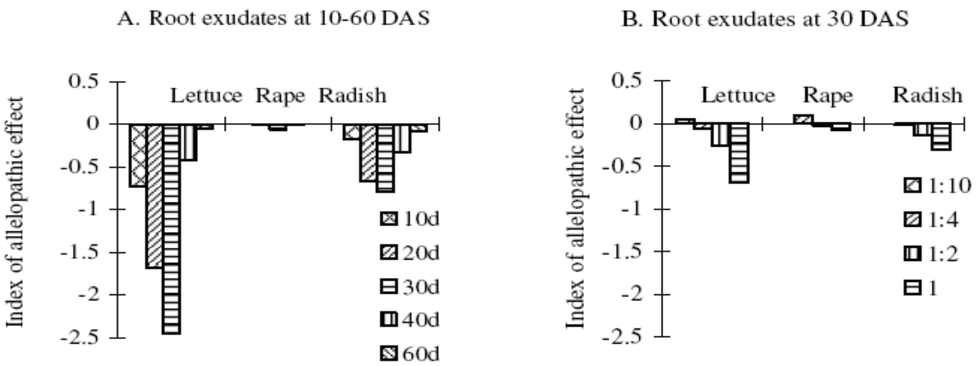

Figure 3. Allelopathic effects of garlic root exudates collected between 10 and 60 days (A) and concentrations collected from 30-day-old garlic plants (B) on test crops.

Seedlings growth

The effects of garlic root exudates on the root and shoot length of test crops seedlings were crop species-dependent (Fig. 4 A). The garlic root exudates from the early growth stages (up to 40-days) stimulated the growth in lettuce and rape. More stimulation in rape was observed with garlic root exudates from 30- and 40-days old plants. The garlic root exudates collected at all the stages inhibited the root length of Radish and the hypocotyl growth of all test crops (Fig. 4 B). Garlic roots exudates were most inhibitory to root growth in radish followed by lettuce and least inhibitory to rape. Contrarily, the garlic root exudates were most stimulatory to shoot growth in rape, followed by lettuce and least stimulatory to radish.

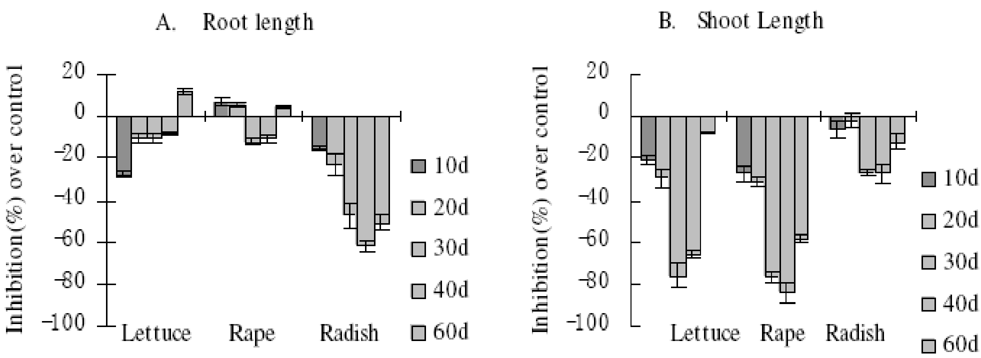

Figure 4. Inhibitory effects of garlic root exudates collected between 10 and 60 days on the root length (A) and shoot length (B) of test crops.

Mycelial growth of *Pythium*

The garlic root exudates collected between 10 -60 days old plants growth were inhibitory (P≤0.05) to mycelia growth of pathogens of peanut (*P. helicoide, P.irregular*, *P. ultimum*) and garlic (*P. violae*, *P. heterothal, P. sylvati*) than untreated (control) mycelia (Fig. 5). The root exudates were more inhibitory to pathogens of garlic than peanut. Plant age had significant effects on the mycelial growth. The garlic root exudates from the early growth stages (10 DAS) significantly inhibited (68.70%-83.74%) the mycelia growth of pathogens of garlic (*P. violae*, *P. heterothal, P. sylvati*), while, root exudates from 20- and 30-day-old plants caused drastic inhibition (84.15-100%) followed by severe inhibition (69.92-89.84%) by root exudates of 40-days old garlic plants The root exudates from 60-day-old garlic plants were least inhibitory to pathogens of both peanut and garlic (Fig. 5 A). The response of peanut pathogens (*P. helicoide, P. irregular, P. ultimum*) was similar to garlic pathogens. However, the inhibitory effects of root exudates from 30 days-old garlic on *P. ultimum* were lower (78.66%) than 40 days-old garlic plants (90.24% (Fig. 5 B).

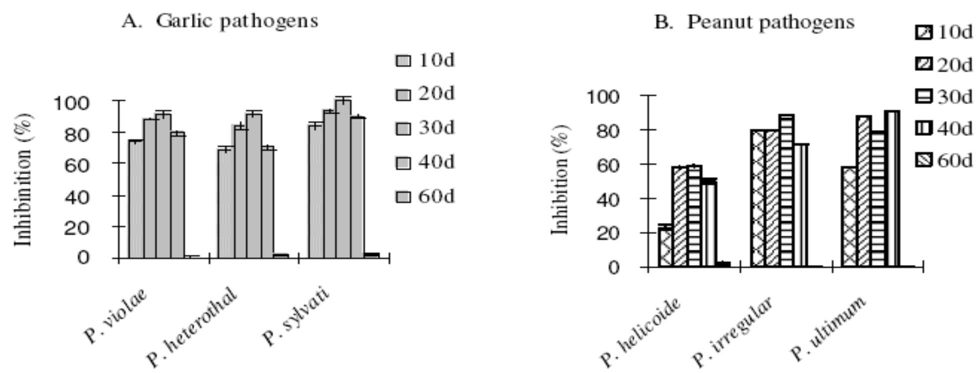

Figure 5. Inhibitory effects of garlic root exudates collected between 10 and 60 days on the garlic and peanut pathogens growth.

At the same plant age, the addition of root exudates to PDA media significantly decreased the mycelial growth (P≤0.05) (Fig 6). At 40-DAS garlic root exudates significantly decreased the mycelial growth of 3-peanut pathogens (*P. helicoide*, *P. irregular* and *P. ultimum*), and the similar trend was observed at 20-DAS garlic root exudates on garlic pathogens (*P. violae, P. heterothal, p.sylvati*). *P. sylvati* proved most sensitive to garlic root exudates among the 6 tested pathogens.

DISCUSSION

Root exudates have allelopathic effects on the seed germination and seedlings growth of crops (1,9,12), but the relationship between plant age and allelopathic effects remains unclear. In this study, the root exudates collected from 10 to 60 days old plants of

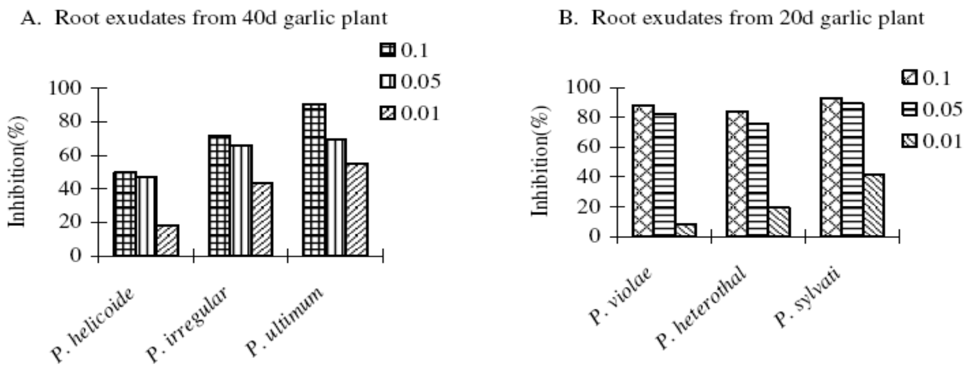

Figure 6. Inhibitory effects of garlic root exudates (40-day-old Plants) on Peanut pathogens (A) and garlic root exudates (20-days-old) on garlic pathogens (B).

garlic, showed allelopathic effects on test crop plants (rape, radish and lettuce). The degree of allelopathic effects depended on the garlic age, root exudates concentration and test crop plant species.

The plant age-dependent effects can be attributed to quantitative and qualitative differences in the secreted allelochemicals due to physiological changes occurring during the plant development. Plant age plays a decisive role in root exudation (4,6). Allelochemicals of root exudates vary during the individual growth stages of garlic (17). Our data indicated that allelopathic effects of root exudates on the crop plants vary with the plant age of garlic and the root exudates from 30-day-old garlic were most potent.

The crop species-dependent effect can be attributed to the diversity of allelopathy in garlic root exudates. The effects of the water extract from garlic are different on various crops (2,16). Our results indicate that allelopathic effects on the lettuce and radish were strong, but were slight on rape.

In our study, we found that RI of root exudates for 3-test crop seeds was positive at lower concentrations, but was negative at higher concentrations. This result is consistent with earlier observations that garlic extracts showed concentration-dependent allelopathic effects on tested crops i.e. suppression at high concentration and promotion at low concentration (14).

Our data also indicated that the garlic root exudates had significant inhibitory effects on the mycelial growth of pathogens from peanut and garlic. Similarly to the tested crops, the degree of inhibition changed with garlic plant age, root exudates concentration and *Pythium* species. Moreover, host plant is the key factor in root exudates-pathogen interactions. In our study, the garlic pathogens (*P. violae*, *P. heterothal, P. sylvati*) were more sensitive to garlic root exudates than the peanut pathogens (*P. helicoide, P. irregular*, *P. ultimum*). The garlic pathogens were highly specialized to garlic, so the possibility of garlic pathogens responses to root exudates from the host plant (garlic) were stronger than the peanut pathogens (*P. helicoide, P. irregular*, *P. ultimum*). It highlights the importance of identifying the antifungal components from the root exudates. Knowledge of the compounds associated with the mechanisms of resistance may aid in our understanding of why it happens and could subsequently be useful in the disease prevention and management.

CONCLUSIONS

The garlic root exudates proved allelopathic to germination and seedlings growth of 3-test crops (Lettuce, rape, radish) and to mycelium growth of 6 pathogens (*P. violae*, *P. heterothal, P. sylvati*, *P. helicoide, P. irregular* and *P. ultimum*). The magnitude of allelopathic effects depended on the garlic plant age and its exudates concentration. Further studies are needed to identify the allelochemicals present in these root exudates, which allelochemicals are most potent and to determine their role in seeds germination and seedlings growth.

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