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Allelochemicals and allelopathy from microorganisms in wheat rhizosphers*

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Abstract—— Allelochemicals from fungi and bacteria in wheat rhizosphere, as well as their allelopathic effects on wheat and maize have been conducted. Fungi and bacteria were incubated at temperature 28－30℃. The extracts were tested for bioassay. Some samples identified to be responsible for allelopathy have been determined by GC-MS. Some chemicals identified were selected to test again their bioactivity. The results showed that acidic extracts were more toxic to germination of wheat and maize seeds (statistic significant difference $P < 0.01$ or $P < 0.05$) and basic extracts were less toxic and stimulated effects on germination in some cases. A good relationship has been observed between the data of chemical analysis and bioassay.

A wide variety of chemicals such as phenolic acids, organic acids, aldehydes, alcohol, ketones, nitrogen-containing chemicals, were identified by GC-MS. Some phenols and organic acids were identified to be inhibit and nitrogens-containing compounds were to be stimulate to germination of seeds and extension of root and shoot of wheat and maize.

Keywords: allelochemicals; allelopathy; soil bacteria; soil fungi.

1 Introduction

After the term allelopathy was coined by Molish in 1937 (Kimber, 1973), the study of allelopathy developed very quickly. James WS *et al.* (James, 1982; Lynch, 1977) reported that the adverse effects are due, at least in part, to a phytotoxic substance produced by the rotting crop residue. There are many examples in the literature that extracts of rotting plant residues were toxic to plant (Hicks, 1989; Thorne, 1990; Kimber, 1967). T. M. McCalla (McCalla, 1964) reported that to "cover the field with 5－10 t/hm^2 wheat straws would inhibited 44%－92% of corn germination. The amount and kind of allelochemicals produced and adverse degree to plants depended on the environmental conditions (Kimber, 1973; Elliott, 1978; Liebl, 1983).

In China, Ma Yongqing and author (Ma, 1993; Zhang, 1994) have also been found inhibition to corn seedling in the field experiments in Hebei and Shandong provinces, when de-

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composition of wheat straw, and soil microorganisms is very important elements in decomposition of wheat straw and wheat residues, but allelochemicals from microorganisms in wheat rhizosphere have never been reported in China.

In this paper, we isolated fungi and bacteria from rhizosphere of Beijing wheat No. 437 and determined allelochemicals and allelopathy by these fungi and bacteria.

2 Materials and method

2.1 Source of soil fungi and bacteria

The soil fungi and bacteria were collected from rhizosphere soil of Beijing wheat No. 437.

2.2 The culture of soil fungi and bacteria

Soil fungi and bacteria were cultured in PDA and general bacteria liquid medium respectively. The medium was sterilized before putting soil fungi and bacteria in, and then 20 mg soil was used as fungi and bacteria, and was put into the 250 ml fungi and bacteria liquid medium respectively. Incubation temperature was controlled on 28—30℃. Liquid medium without soil was used as control.

2.3 Extraction of secretion of soil fungi and bacteria

The samples were taken out, after incubation: 3 days, 1 week, 2 weeks and 4 weeks. First the samples were filtered through Whatman No. 3 filter paper. The acidic fractions (fungi (A), bacteria (A)) were obtained by acidifying the filtrate to pH 2 with 1 mol/L HCl and extracting two times with 50 ml CH_2Cl_2. The basic fraction (fungi (B), bacteria (B)) were obtained in a similar manner by adjusting the acidified residues to pH 12 with 1 mol/L NaOH and extracting two times with 50 ml CH_2Cl_2. The fractions were dried over anhydrous MgSO4 and concentrated to a final volume of 1 ml in a rotary evaporator at 40—50℃. As 0.5 ml of the acidic and 0.5 ml of basic concentrates were taken out from above 1 ml and were diluted with distilled water to 10 ml for bioassay. The remaining concentrates of both fractions were used for further analysis by GC-MS.

2.4 Bioassay

As 3 ml and 5 ml of acidic and basic fractions were applied separately on 8 cm and 12 cm diameter of Whatman No. 3 filter papers placed in 9 cm and 13 cm diameter glass dishes. The seeds of wheat and maize were presoaked for 4 and 8h respectively before test. 10 wheat seeds and 20 maize seeds were placed on the filter paper respectively. The wheat and maize seeds were obtained from the Chinese Academy of Agriculture Science. Germination was carried out in a moisture-saturated dark chamber for 48h at 28—30℃ and measured the length of the roots and shoots. All treatments were performed in triplicate, and the allelopathic effects on the germination, root and shoot were expressed as percentage of inhibition and stimulation. Both distilled H_2O and concentrated CH_2Cl_2 were used as control. The data were analyzed by biostatistic.

2.5 Analysis by GC-MS

GC model HP5890, equipped with FID and 30m (0.25 mm I.D.) DB-5 fused silica cap-

illary column (95% dimethyl—5% diphenylpoly siloxane; J & W Scientific, Inc, Rancho cordova, CA). Injection port temperature 240℃; detector temperature 280℃; initial column temperature 50℃; held for 2 min and then programmed to 275℃ at 6℃/min; carrier gas N_2; flow rate 1 ml/min injected volume 0.001 ml. Another column was carbowax (0.25 mm × 30 cm), injection port temperature 240℃; detector temperature 275℃; initial column temperature 50℃, held for 2 min and then programmed to 250℃ at6℃/min.

GC-MS was performed on Model TR102000. All conditions except carrier gas He were the same as GC. Electron impact (EI), scan range M/Z 30-600AMU, scan rate 0.2 second; ion source temperature 150℃. The unknown compounds were identified by computer library search system.

3 Results

3.1 Influences of fungi (A,B) and bacteria (A,B) to root and shoot length of wheat and maize

The bioassay results are shown in Fig. 1—4. It was observed that extract of bacteria (A) and fungi (A) samples for a period incubation 3 days have shown a stronger inhibition ($P<0.01$) to elongation of wheat and maize root. The wheat root length was shortened

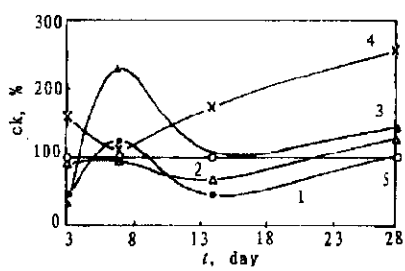

Fig. 1 Influence of bacteria (A), bacteria (B), fungi (A) and fungi (B) to wheat root length
1: bacteria (A) 2: bacteria (B) 3: fungi (A)
4: fungi (B) 5: ck

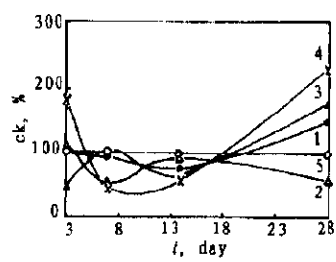

Fig. 2 Influence of bacteria (A), bacteria (B), fungi (A) and fungi (B) to wheat shoot length
1, bacteria (A) 2, bacteria (B) 3, fungi (A)
4: fungi (B) 5: ck

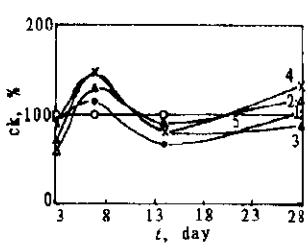

Fig. 3 Influence of bacteria (A), bacteria (B), fungi (A) and fungi (B) to maize shoot length
1: bacteria (A) 2: bacteria (B) 3: fungi (A)
4: fungi (B) 5: ck

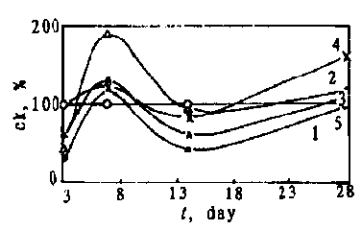

Fig. 4 Influence of bacteria (A), bacteria (B), fungi (A) and fungi (B) to maize root length
1: bacteria (A) 2: bacteria (B) 3: fungi (A)
4: fungi (B) 5: ck

50%—60% in compare with the control. The samples of fungi (B) was unusual, because it stimulated the growth of wheat root and shoot ($P<0.01$). Most samples could stimulate the growth of wheat (maize) root (shoot) in incubation one week, but after two weeks, almost all of samples could inhibit the growth of wheat (maize) root (shoot) except the sample fungi (B). After four weeks, all of samples had stimulation again. The stimulation to wheat in all of samples was more than to maize.

3.2 Identification of unknown compounds in samples by GC-MS

The compounds identified by GC-MS are given in Table 1. The best library matches coefficient in the sample and standard spectra by data system compounds listed should be > 700. Different kind of chemicals, such as phenolic acids, organic acids, aldehydes, alcohols, ketones and esters etc, were identified in the samples of fungi (A) and bacteria (A). The another kind of chemicals such as nitrogen containing compounds have been identified in the samples of fungi(B) and bacteria (B).

Table 1 Chemical materials were produced by fungi and bacteria with GC-MS analysis *

Fungi (A)	Bacteria (A)	Bacteria (B)
Dimethyl-propanedioic acid	Dimethyl-propanedioic acid	4-methylphenol
Phenyl-propanedioic acid	Phenyl-propanedioic acid	Dimethylhydrazone, valeral dehyde
4-methyl-acetic acid	Benzenepropanoic acid	2-methyl-benzonitrile
E-2-butanacenoic acid	4-methyl-pentanoic acid	2,7-methyl-1,7-octadien-3-amine
9-hexadecenoic acid	4-methyl-heptanoic acid	1-methylpiperidine
1-Ethyl-4-methylbenze		Tricaaarbonyl N-(pheny-2-pyridinylmethylene benzenamine
1-Ethyl-1H-indene	Dumethylhydrazone vaieraldehyde	3-methyl-(Z)-2-decene
1,2-benzisothiazole	1-Ethyl-3-piperidinol	1,2-Dimethyl-3-pentyl-cyclopropane
3-(1-methylbutoxy)-2-butanol	2,7-Dimethyl-1,7-octadien-3-amine	2,4,6-Triethyl-5-methyl-1,3,2-dioxaborinane
1,3-Butanediol	6-Ethyl-2-methyl-decane	3,3,5,5-Tetrametmyl,1.2-cyclopentanedione
2-Propenoic acid,2-methyl-propyl ester	4-methyl-heptanoic	
Butanamide	Tetrahydro-6-propyl-2H-pyran-2-one	
5-Heptyldihydro-2(3H)-furanone	2,4-Imidazolidinedionel-methyl	
Tetrahydro-6-propyl-2H-pyran-2-one		

3.3 Bioassay results for same compounds identified by GC-MS

Many compounds were identified by GC-MS. Some compounds were for bioassay again, make further verifiable toxicity of some secretion of fungi and bacteria on growth of crops. We selected 10 ethyl-3-piperidinol, 4-methylphenol and benzenepropanoic acid. The results are shown in Table 2. It could be seen that more than 40% and 15%—30% of root length on wheat and maize were inhibited by 4-methylpgenol and benzenepropanoic in concentration

200 ppm compared with control, and 30% 40% and more than 40% of shoot length of wheat and maize also were inhibited by them. 15%—30% and 30%—40% of root length of wheat and maize were inhibited by 200 ppm of 1,3-butanediol. More than 40% of shoot length of wheat and maize also were inhibited by 1,3-butanediol.

Table 2 Bioassay results for some compounds identified by GC-MS

Chemicals	Concentration,	Wheat		Maize	
	ppm	Root	Shoot	Root	Shoot
1,3-butanediol	200	++	++++	+++	++++
	500	++	++++	+++	+++
4-methylphenol	200	++++	++++	++++	++++
	500	++++	++++	++++	++++
Benzenepropanoic acid	200	+	+++	++++	++++
	500	++++	+++	++++	++++

+ inhibit 15% of root and shoot length ++ inhibit 15%—30% of root and shoot length +++ inhibit 30%—40% of root and shoot length ++++ inhibit more than 40% of root and shoot length

4 Conclusion

A wide variety of toxic chemicals were produced by fungi and bacteria in rhizosphere of Beijing Wheat No. 437. The effects of the various extracts of fungi (A,B) and bacteria (A, B) on the elongation of roots and shoots of wheat and maize varied considerably. The maximum inhibition period of allelochmicals was about 2 weeks. The toxic effect is normally of a disappearing after four weeks. Kimber (Kimber, 1967) and Zhang Yumin (Zhang, 1994) reported that maximum inhibition occurred after 2—10 days. The results is important for next planting.

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