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The allelopathy research of the endophytic fungi's secondary metabolite in pleioblastus amarus(keng)keng f.

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ABSTRACT

This paper reveals the allelopathy on the following three crops, brassica campestris L, Raphanus sativus L and Lactuca sativa through studying the seed germination and the seedling growth of the five different elution parts (marked part a, b, c, d, e) produced by the secondary metabolites of the Xylaria sp.(LI). Makes the first endophytic -fungi separation for the (Pleioblastus amarus (Keng) Keng f.) grown on the campus of Zhejiang University of Agriculture and Forestry; obtained the fungi assayed to be Xylaria sp.(L1) by adopting the methods of morphological observation and molecular biology;separate and obtained its secondary metabolites after making a liquid fermentation for separated Xylaria sp.(L1); Use the methods of culture dish and cuvette to study the influence of different parts of the secondary metabolites in Xylaria sp.(L1) on the seed germination and seedling growth of the following three crops (Brassica campestris L.), (Raphanus sativus L), and (Lactuca sativa). Different extract parts of Xylaria sp.(L1) and its secondary metabolites have double concentration effects, that is, "Low concentration promotion but high concentration inhibition" on the germination rate and germination index of the two crops (Raphanus sativus L), and (Lactuca sativa), with no influence on that of (Brassica campestris L.); High density Xylaria sp. (LI) and its secondary metabolites have a clear inhibiting effects on the root-length, seedling height and the raw weight of the three crops (Brassica campestris L.), (Raphanus sativus L), (Lactuca sativa), while the a,b,c parts of the low-density Xylaria sp. (LI) and its secondary metabolites have a clear facilitating effect on the root-length, seedling height and the raw weight of (Lactuca sativa); the c part of the low-density culture solution also has a clear facilitating effect on the root-length, seedling height and the raw weight of (Brassica campestris L.). In a certain concentration, the Xylaria sp. (LI) and its secondary metabolites both have clear inhibiting effects on the seed germination and seedling growth of the three crops, and the higher the concentration of the culture solution, the stronger the inhibiting effect.

KEYWORDS

Endophytic fungi in pleioblatus amarus; *Xylaria sp.*(L1) fungi; Secondary metabolite; Seed germination; Seedling growth; Allelopathy.

INTRODUCTION

(*Pleioblastus amarus (Keng) Keng f.*) is a species of the Pleioblastus genus in grass family. This plant is mainly grown in the southern provinces of China[1-3]. (*Pleioblastus amarus (Keng) Keng f.*)is an excellent bamboo with dual purpose of shoot and timber, and its shoots can be edible and used as medicine;bamboo wood is an excellent paper-making material[4,5]. Therefore, developed reasonbly, it can benefit a lot for the local farmers. The endophytic fungi refers to the fungi living in the inner part of the plant tissues in a certain period of time during its life, and cause no clear disease symptoms for the plants[6-9]. It is a kind of microorganism living in the inner part of the plant tissues and it is an inseparable natural components of the plant micro-ecosystem[10]. The research shows that if the endophytic fungi lives in the plants for a long time, it can produce a secondary metabolites having the similar or even the same bioactivity as those in their host plants. These secondary metabolites have important practical values in medicine, agriculture and the prevention of the plant diseases and insect pests[11-15]. In China where the plant resources are overused and the environment is deteriorating, as a new kind of not-fully developed and researched micro-organism resources, the endophytic fungi has a very great potential applicable prospect[16].

This paper uses fresh (*Pleioblastus amarus (Keng) Keng f.*) as the material, isolates and determines a Xylaria sp.(LI) fungus, and ferments it in a large amount, meanwhile extracts and separates its fermentation products, and obtains the secondary metabolites in different extracted parts. The paper mainly researched the influence for the germination and seedling growth of the four agricultural crops of the secondary metabolites in the five different extracted parts in the endophytic fungi *Xylaria sp.*(L1), and reveals that the secondary metabolites of endophytic fungi *Xylaria sp.*(L1) has the allelopathic effects on the three crops*Brassica campestris L., Raphanus sativus L, Lactuca sativa* and this will provide some scientific data for the reasonable development of *Xylaria sp.*(L1).

EXPERIMENTAL

Materials and methods

The testing material (*Pleioblastus amarus* (*Keng*) *Keng f.*) is picked from the campus of Zhejiang University of Agriculture and Forestry; the acceptor materials are the local crops (*Brassica campestris L.*), (*Raphanus sativus L*), (*Lactuca sativa*) and the seeds are bought from the Lin An Seed Company.

The separation and determination of the endophytic fungi *xylaria sp.*(L1)

The bacterial strain L1 comes from the fresh bitter bamboo (*Pleioblastus amarus (Keng) Keng f.*) in Zhejiang University of Agriculture and Forestry, and determined as a strain of *Xylaria sp* after the morphological observation and molecular biology. The extraction of the fermentation products in *Xylaria sp.* (L1) fungi: First, ferment the bacterial strains of L1, and after the fermentation, filter the fermentation liquid with gauze and obtain the liquid and mycelia, respectively. Use the big-hole resin to filter the bacterial liquid. First wash it with water and then use methyl alcohol to wash till colorless and collect the methanol flushing fluid and get concentrated solution LIA by vacuum concentration;Put the mycelia on the gauze and dry in the oven at the temperature of 50℃. Place the dried mycelia and the gauze into the methanol liquid and after supersonic extraction directly concentrate it by vacuum concentration method, we will then get the methanol extracted concentration liquid of mycelia L1B;combine the concentrated solutions of L1A and L1B, firstly, use the petroleum ether to extract, obtaining the petroleum ether extracted solutin, and then use ethyl acetate extraction liquid and get the extract of L1C by vacuum extraction method.

The preparation of the mother solution of the allelopathy experiment and the culture solution

Separate the extract L1C with silicagel column and make a gradient elution by using the respective ratios of dichloromethane : methynol =100:0 ; 90:10 ; 80:20 ; 60:40 ; 50:50, and get the five corresponding elution parts a,b,c,d,e and then concentrate them to dried powder by vacuum extraction method.

Treatment for the receptor seeds

The receptor seeds are all disinfected for 40 min by using 1% NaClO; use running water to wash 2～3 times, and then use the distilled water to wash it clean, and then dry it for later use.

The determinatio of the seed germination

Adopt the Culture-dish Filtration Method to do the experiment of seed germination. Take the prepared culture solution in 1.2.3, and put 5ml of each one into the culture dish deckened with 2 layers of filtration paper; when the solvent of the culture solution volatized and dried, put 5ml distilled water into each dish, and then put the even-sized, plump receptor seeds into the culture dish, 50 grains in each dish. Then, place the culture dishes into the culture oven at constant temperature of 25℃ to cultivate in the dark, with three times of repetition for each dish and use distilled water as the control group. Record the germinated number of the receptor seeds every 24h, with the germination standard that the radicle or hypocotyl break the seed skin of 1～2 mm in length, and at the same time calculate the seed germination rate and the germination index.

The determinatio of the seedling growth

Adopt the Cuvette Method[17] to make the biological seedling determination. Pave two layers of filtration paper in the bottom of the 100ml flask, and put the prepared culture solution in 1.2.3 into the beaker respectively, and use distilled water as the control group, and then, put 5 receptor seedlings which are all pre-sprouted and with equal good growth, and use

sealing film to seal up the cup-mouth for preventing water evaporation and the interplay effects between different treatments. Put the small beakers in the 12h/d oven with constant temperature 25°C, each treatment having 3 repetitions and on the sixth day, determine the root length, seedling height and the fresh weight..

Data statistics and analytical methods

The seed germination rate GR= ΣGt / T×100%,in this equation, Gt represents the total germinating number in t days, T is the total seed number. The seed germination index GI =Σ(Gt / Dt),in this equation, Gt represents the germination number on the tth day, Dt represents the corrresponding germination days. The allelopathic effect index (RI) can be obtained according to the Williamson equation[18]. RI=1- C /T (T≥C) RI=T / C- 1 (T<C),in this equation, C stands for the control value, T is the treatment value, when RI > 0, it shows the facilitating effect of the allelopathy, when RI< 0, it shows the inhibitory effect of the allelopathy, and the absolute value of RI shows how strong the allelopathy. Analyze the experiment data by SPSS17.0 and Excel, plot by using the software oringin8.0.

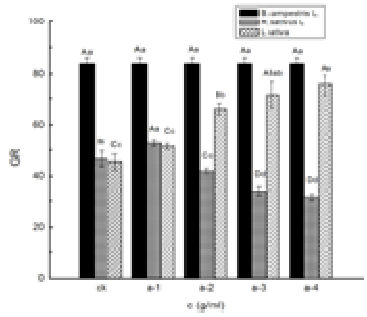
RESULTS AND DISCUSS

The influence of the secondary metabolites in the endophytic fungi *Xylaria sp.*(L1) on the receptor seed germination

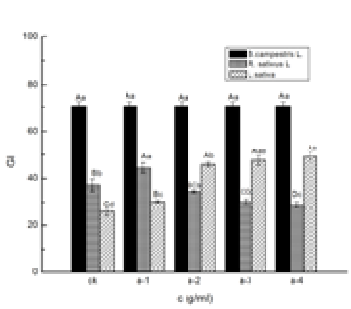
The secondary metabolites of endophytic fungi *Xylaria sp.*(L1) has influence on the seed germination rate and germination index of the receptor and this can be seen in Figure 1. The result shows that the different extracting parts of the secondary metabolites of endophytic fungi *Xylaria sp.*(L1) have the double concentration effects of so-called "promoting with low density, but inhibiting with high density", but there are differences in different seeds.

For the receptor seeds of *Raphanus sativus L*, compared with the control group, the culture liquid began to inhibit the seed germinaiton, and the higher the density of the culture liquid, the stronger the inhibitory effect.

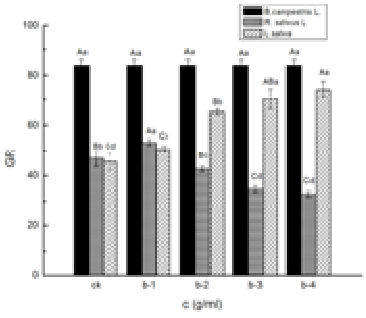
For the receptor seeds of *Lactuca sativa*, the a,b,c parts of the culture liquids all have the promoting effects on the seed germination of *Lactuca sativa*; For the receptor seeds of *Brassica campestris L*, all the treatment liquids have no influence on its seed germination.



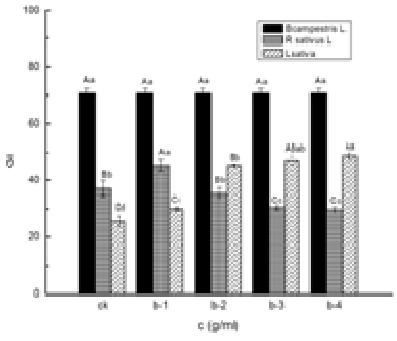
Germination rate of part a



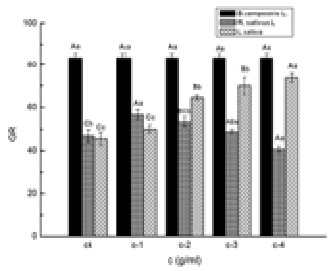
Germinaiton index of part a



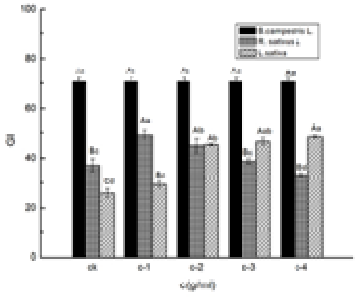
Germination rate of part b



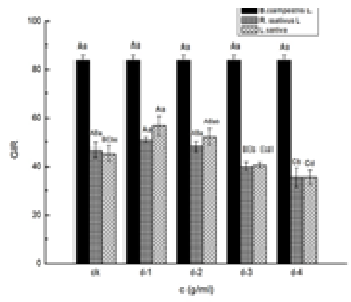
Germinaiton index of part b



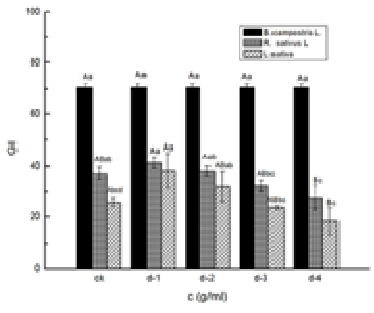
Germination rate of part c



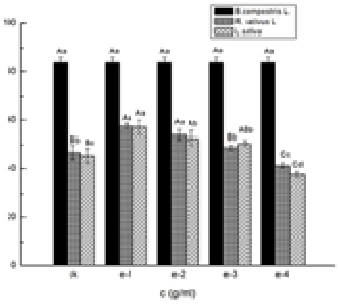
Germinaiton index of part c



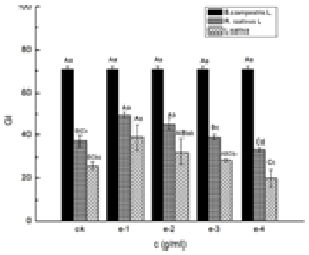
Germination rate of part d



Germinaiton index of part d



Germination rate of part e



Germinaiton index of part e

Figure 1 : The influence of part "a-e" on the germination rate and germination index

The Influence of the Secondary Metabolites of Endophytic Fungi *Xylaria sp.* (L1) on the Crop Seedling Growth: The influence on the growth of seedling root-length and seedling height see TABLE 1-5.

TABLE 1 : The influence of "part a" on the crop seedling root-length and sprout height

Result of analysis	Concentration g/ml	Root-length X±S.E(cm)	RI	Sprout height X±S.E(cm)	RI
B.campestris L.	ck	7.0833±0.1358Aa	-	5.6600±0.1539Aa	-
	a-1	7.0800±0.1442Aa	-0.0004	5.6600±0.1710Aa	0
	a-2	7.0767±0.1222Aa	-0.0009	5.6500±0.1242Aa	-0.0018
	a-3	7.0733±0.1351Aa	-0.0014	5.6300±0.151Aa	-0.0053
	a-4	7.0725±0.1253Aa	-0.00015	5.6213±0.1430Aa	-0.0068
R.sativus L	ck	8.4533±0.0179Aa	-	5.0233±0.0777Aa	-
	a-1	5.5133±0.1644Bb	-0.3478	4.5700±0.2070Bb	-0.0902
	a-2	4.1967±0.1762Cc	-0.5035	2.3500±0.0436Cc	-0.5322
	a-3	3.7700±0.1127Dd	-0.5540	2.216±0.0473CDc	-0.5587
	a-4	3.3233±0.0681Ee	-0.3931	1.940±0.1229Dd	-0.6138
L.sativa	ck	4.1000±0.1000Bb	-	3.7800±0.0265Bb	-
	a-1	4.5367±0.0551Aa	0.0963	4.0633±0.0550Aa	0.0697
	a-2	3.7733±0.2053Cc	-0.0797	3.5433±0.1626Cc	-0.0626
	a-3	3.4133±0.0153Dd	-0.1675	3.4400±0.0100Cc	-0.0899
	a-4	2.9167±0.1047Ee	-0.2886	3.1033±0.1002Dd	-0.1790

TABLE 2 : The influence of "part b" on the crop seedling root-length and sprout height

Result of analysis	Concentration g/ml	Root-length X±S.E(cm)	RI	Sprout height X±S.E(cm)	RI
B.campestris L.	ck	7.0833±0.1358Aa	-	5.6600±0.1539Aa	-
	b-1	5.2533±0.1858Bb	-0.2583	4.5133±0.0586Bb	-0.2026
	b-2	3.2967±0.0839Cc	-0.5346	3.5367±0.0321Cc	-0.3751
	b-3	2.3700±0.0608Dd	-0.6654	2.7700±0.0608Dd	-0.5106
	b-4	0.4200±0.1058Ee	-0.9407	2.4533±0.0503Ee	-0.5666
R.sativus L	ck	8.4533±0.0179Aa	-	5.0233±0.0777Aa	-
	b-1	6.2533±0.0503Bb	-0.2603	4.3533±0.0503Bb	-0.1334
	b-2	5.1700±0.0608Cc	-0.3884	4.0800±0.0436Cc	-0.1879
	b-3	4.1333±0.0651Dd	-0.5110	3.9667±0.0306Cd	-0.2103
	b-4	2.5900±0.0361Ee	-0.6936	3.5000±0.0300De	-0.3032
L.sativa	ck	4.1000±0.1000Aa	-	3.7800±0.0265Aa	-
	b-1	4.6033±0.0950Bb	0.1091	4.1500±0.0954Bb	-.0.8900
	b-2	3.8733±0.1419Bc	-0.0553	3.6100±0.1054Cc	-0.0449
	b-3	3.4233±0.0321Cd	-0.1650	3.4433±0.0153Cd	-0.0891
	b-4	2.9367±0.1184De	-0.2837	3.1433±0.1250De	-0.1684

TABLE 3 : The influence of "part c" on the crop seedling root-length and sprout height

Result of analysis	Concentration g/ml	Root-length X±S.E(cm)	RI	Sprout height X±S.E(cm)	RI
B.campestris L.	ck	7.0833±0.1358Aa	-	5.6600±0.1539Aa	-
	c-1	7.6000±0.2650Bb	-0.0680	5.9133±0.1050Bb	0.0428
	c-2	4.6300±0.1127Cc	-0.3464	4.1400±0.0954Cc	-0.2686
	c-3	4.0067±0.0493Dd	-0.4257	3.7167±0.1041Dd	-0.3433
	c-4	2.3800±0.1058Ee	-0.6640	3.3833±0.1040Ee	-0.4022
R.sativus L	ck	8.4533±0.0179Aa	-	5.0233±0.0777Aa	-
	c-1	6.3667±0.1514Bb	-0.2468	4.4767±0.1686Bb	-0.1088
	c-2	5.2233±0.0321Cc	-0.0422	4.1800±0.1300Cc	-0.1679
	c-3	4.2067±0.0802Dd	-0.5024	4.0167±0.0666Cc	-0.2004
	c-4	2.6400±0.0529Ee	-0.6877	3.5700±0.0964Dd	-0.2893
L.sativa	ck	4.1000±0.1000Bb	-	3.7800±0.0265Bb	-
	c-1	4.6433±0.0513Aa	0.1170	4.1700±0.0819Aa	0.0935
	c-2	3.9300±0.0608Bb	-0.0415	3.6367±0.0713Bc	-0.2392
	c-3	3.4333±0.0252Cc	-0.1626	3.4500±0.0100Cd	-0.0873
	c-4	3.0033±0.2000Dd	-0.2682	3.2100±0.1050De	-0.1508

TABLE 4 : The influence of "part d" on the crop seedling root-length and sprout height

Result of analysis	Concentration g/ml	Root-length X±S.E(cm)	RI	Sprout height X±S.E(cm)	RI
B.campestris L.	ck	7.0833±0.1358Aa	-	5.6600±0.1539Aa	-
	d-1	5.1367±0.0551Bb	-0.2748	4.4800±0.0100Bb	-0.2085
	d-2	3.2500±0.0781Cc	-0.5412	3.5200±0.0265Cc	-0.3781
	d-3	2.3367±0.0553Dd	-0.6701	2.7401±0.0529Dd	-0.5159
	d-4	0.3333±0.1528Ee	-0.9529	2.4033±0.0950Ee	-0.8233
R.sativus L	ck	8.4533±0.0179Aa	-	5.0233±0.0777Aa	-
	d-1	6.2167±0.0379Bb	-0.2646	4.3133±0.0416Bb	-0.1413
	d-2	5.1367±0.0551Cc	-0.3923	4.0567±0.0379Cc	-0.1924
	d-3	4.0933±0.0321Dd	-0.5158	3.9467±0.1550Cd	-0.2143
	d-4	2.5400±0.0529Ee	-0.6995	3.4433±0.0379De	-0.3145
L.sativa	ck	4.1000±0.1000Bb	-	3.7800±0.0265Bb	-
	d-1	3.9800±0.0608Aa	-0.0293	3.6833±0.0551Aa	-0.0256
	d-2	3.7733±0.1102Bb	-0.0797	3.5333±0.0586Bb	-0.0653
	d-3	3.3700±0.0608Cc	-0.1780	3.4233±0.0208Cd	-0.0944
	d-4	2.8700±0.1127Dd	-0.3000	3.1100±0.0954De	-0.1772

It is known for Figure 1, in all the above statistics, all the allelopathy index are all the negative values except that the allelopathy index RI of the low-density a,b,c parts in Lactuca sativa is greater than zero after treating its culture liquid, and this shows that the secondary metabolites of endophytic fungi *Xylaria sp.* (L1) has both inhibitory and promoting effects on the crop seedling root-length and seedling height, and its inhibitory effect is the most, and its inhibitory effects are higher as the density increases. The different crops have different reactions to different treating fluids.

For the seedlings of *Brassica campestris L*, a-part has no significent influence on its seedling root-length and seedling height; the low-density of c-part has the promoting effect on the seedling root-length and seedling height of *Brassica campestris L*; the high-density culture liquids of b,c,d,e parts have clear inhibitory effects on the seedling root-length and seedling height of *Brassica campestris L*, and the inhibitory effects are more obvious with the increasing of the culture liquid density, especially when the density of the b,d parts are b-1,d-1(1.28×10^{-3}g/ml) respectively,the seedling root growth of *Brassica campestris L* almost stop growing and the roots begin to soften and go mouldy and blacken.

For the seedlings of *Raphanus sativus L*, all the treatment liquids have clear inhibitory effects on its root-length and seedling height, and the inhibitory effects are more obvious with the increasing of the culture liquid density.

For the seedlings of *Lactuca sativa*, the low-density of a,b,c-part has the promoting effects on the seedling root-length and seedling height of *Lactuca sativa*; all the other treatment liquids have inhibitory effects on its root-length and seedling height.

TABLE 5 : The influence of "part e" on the crop seedling root-length and sprout height

Result of analysis	Concentration g/ml	Root-length X±S.E(cm)	RI	Sprout height X±S.E(cm)	RI
B.campestris L.	ck	7.0833±0.1358Aa	-	5.6600±0.1539Aa	-
	e-1	6.9800±0.1217	-0.0146	5.5667±0.1850	-0.0165
	e-2	6.4933±0.0704	-0.0833	5.1467±0.0473	-0.1084
	e-3	5.4400±0.0529	-0.2320	4.4467±0.0473	-0.8233
	e-4	3.2033±0.0252	-0.5478	2.4467±0.0945	-0.5642
R.sativus L	ck	8.4533±0.0179Aa	-	5.0233±0.0777Aa	-
	e-1	5.4133±0.0321Bb	-0.3596	4.4433±0.0586Bb	-0.1155
	e-2	4.2367±0.1518Cc	-0.4988	2.36±0.0361Cc	-0.5302
	e-3	3.8000±0.1120Dd	-0.5505	2.2367±0.0351Cc	-0.5547
	e-4	3.2933±0.0208Ee	-0.6104	1.9333±0.1159Dd	-0.6151
L.sativa	ck	4.1000±0.1000Aa	-	3.7800±0.0265Aa	-
	e-1	3.9933±0.037ABa	-0.0260	3.6900±0.0436Aa	-0.0238
	e-2	3.8067±0.1007Bb	-0.0715	3.5433±0.0551Bb	-0.0626
	e-3	3.4100±0.0100Cc	-0.1683	3.4433±0.0153Bc	-0.0891
	e-4	2.8800±0.1044Dd	-0.2976	3.1100±0.0954Cd	-0.1772

The influence on the fresh weight of the seedlings

The influence of the secondary metabolites of endophytic fungi *Xylaria sp.* (L1) on the seedling fresh weight is almost the same with the influence on its root-length or seedling height.The variation trend of the seedling fresh weight in *Lactuca sativa)* is the smallest, this may show the low-sensitivity for the allelochemicals contained in the secondary metabolites of endophytic fungi *Xylaria sp.* (L1) in Lactuca sativa; while the variation trend of the seedling fresh weight in *Raphanus sativus L* is the biggest, this may show the high-sensitivity for the allelochemicals contained in the secondary metabolites of endophytic fungi *Xylaria sp.* (L1) in *Raphanus sativus L;* however, the variation trend of the seedlings in Brassica campestris L is comparatively moderate.

CONCLUSIONS

The allelopathy is the direct or indirect, advantageous or disadvantageous effect produced during the metabolic process of plants or micro-organisms[19,20]. The test results show that the secondary metabolites of endophytic fungi *Xylaria sp.* (L1) have inhibitory or promoting effects on the seed germination and seedling growth of the three crops, this shows that there is allelochemical in the secondary metabolites of endophytic fungi *Xylaria sp.* (L1).

The allelochemicals in the secondary metabolites of endophytic fungi *Xylaria sp.* (L1) have different influence on the different receptor seeds and seedlings; the leaching liquid of the same part and same density have the influence more sensitive on *Raphanus sativus L and Lactuca sativa* than on *Brassica campestris L*; the allelochemicals produced in the different parts of the secondary metabolites of endophytic fungi *Xylaria sp.* (L1) are not the same, either, that is, different culture liquids have different influence on the seeds and seedlings of the same receptor.

The allelochemicals in the secondary metabolites of endophytic fungi*Xylaria sp.* (L1) can dissolve into the soil through rain water, and thus interfere with the growth of the plants in the neighboring places. So how to properly develop and make use of *Xylaria sp.* (L1) fungi needs further research.

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