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ALLELOPATHIC EFFECT OF METABOLITES PRODUCED BY *STREPTOMYCES SP*. HU2014 ON WHEAT AND GREEN BRISTLEGRASS

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Plant growth-promoting rhizobacteria widely exist in the plant rhizosphere. They provide nutrition and produce some antibiotic substances to suppress the plant diseases and promote the growth of plants. Therefore, the study of allelopathy is a very important part in the interaction between rhizobacteria and plant. Streptomyces, a genus of actinomycetes, is well known for its bioactive metabolites, mainly including antibiotics, hormones, and hydrolase, which can affect plants growth. In the present study, the allelopathic effect of Streptomyces sp. HU2014 metabolites on wheat (Triticum aestivum L.) and green bristlegrass (Setaria viridis (L.) Beauv.) were estimated using the response index (RI). Negative RI values indicated inhibition of plant growth; positive values indicated stimulation of plant growth. Four fractions (F2, F4, F6 and F8) from the cell-free filtrates of HU2014 culture broth had a certain effect on the shoot and root length of above tested plant seedlings. For wheat, the results showed that fraction of F2 at 10 mg/ml had the strongest inhibition on the shoot length (RI = -0.53) and root length (RI = -0.22). However, fraction of F2 at 1 mg/ml promoted the shoot length (RI = 0.01). Fraction of F4 and F6 at 10 mg/ml had strongest inhibition on the shoot and root length. Fraction of F8 had the highest inhibition on the shoot length (RI = -0.66) at 5 mg/ml, and the root length (RI = -0.66) at 10 mg/ml. For green bristlegrass, F2 fraction at 10 mg/ml had the strongest inhibition on the shoot (RI = -0.73) and root length (RI = -1.00). F4 fraction had the highest inhibition on the shoot length (RI = -0.69) at 5mg/ml, and the root length (RI = -0.85) at 10 mg/ml. Fraction of F6 had the highest inhibition on the shoot length (RI = -0.59) at 10 mg/ml, and the root length (RI = -0.80) at 5 mg/ml. Fraction of F8 had the highest inhibition on the shoot length (RI = -0.47) at 5mg/ml, and the root length (RI = -0.93) at 10 mg/ml. From the above results, we can draw a conclusion that four fractions had the allelopathic effects on the shoot and root length of two tested plants except F2 fraction at 1 mg/ml promoting the wheat shoot length. Thus, at an early stage of plant growth, the low concentration of allelopathic substances produced by HU2014 may promote the growth of wheat, while these agents inhibit the growth of green bristlegrass. Therefore, this strain can be promising both as a biofungicide and as a bioherbicide.

Key words: *Streptomyces, allelopathy, response index, wheat, green bristlegrass.*

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Introduction. Allelopathy is a common biological phenomenon by which one organism produces biochemicals that influence the growth, survival, development, and reproduction of other organisms. Animals, plants and microorganisms are among them. These biochemicals are known as allelochemicals and have beneficial or detrimental effects on target organisms (Cheng et al., 2015). Therefore, allelopathy is accepted as a technology to implement for weed control and biological control of other useful traits (Ozcatalbas et al., 2010).

Allelochemicals are released into the soil rhizosphere by a variety of mechanisms, including metabolites, decomposition of residues, and exudation. Moreover, these chemicals play a role in plant defense, nutrient chelation, and regulation of soil biota (Aslam et al., 2017). The allelochemicals found so far are mostly secondary metabolites of plants or microorganisms. Organic acids with low molecular weight, phenols and terpenoids are most common.

Volatile organic compounds (VOCs) have the allelopathic effects on plant physiological and biochemical processes including growth, content of reactive oxygen species (ROS), enzyme activity, and photosynthesis except plant-to-plant communication as a signaling substance (Xie et al., 2021).

Sorgoleone separated from *Sorghum* had the potential for weed control and yield enhancement (Hussain et al., 2021). Three chemicals – veratric acid, maltol, and (-) loliolide,- were isolated from crabgrass significantly inhibited the growth of wheat, maize, and soybean and reduced soil microbial biomass carbon (Zhou et al., 2013). Cinnamic acid and vanillin in eggplants root exudates changed the microbes population of grafted eggplants (Chen et al., 2011).

However, there are relatively few reports on the allelopathy of microorganisms which also play an important role in the biological chain of ecosystem. It was reported 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-Phenol and 1,2,3,4-Butanetetrol identified from *Bacillus amyloliquefaciens* inhibited the seeds germination of *C. equisetifolia* (Chen et al., 2021).

Streptomyces can produce a variety of bioactive substances, which play an important role in improving the plant disease resistance and promoting growth (Katz et al., 2016; Tarkka et al., 2008). Therefore, this species is promising in agriculture as plant-growth-promoting (PGP) bacteria and/or biological control agents (BCAs) (Dias et al., 2017; Viaene et al., 2016). However, in practical application, we should also consider the impact of it on cash crops and weeds in field.

Long-term research is devoted to the study of the effectiveness of biological control of plant diseases by strains *Streptomyces sp.* HU2014. In view of the interactions between the strain and plants, it is necessary to research the allelopathy of this strain on wheat (*Triticum aestivum* L.) and weeds. In this study, green bristlegrass (*Setaria viridis* (L.) Beauv.), one of weeds, was chosen due to its strong adaptability and widespread distribution in farmland in China. Four fractions of the cell-free filtrates of *Streptomyces ap.* HU2014 culture broth were applied to assess the allelopathic effect on the seed germination of wheat and green bristlegrass. Four fractions from the fermentation broth of HU2014 were co-cultured at different concentration with the seeds of two plants and the sensitivity index of them was detected.

Materials and methods. The experiment was conducted in March 2021 in Xinxiang, China. *Streptomyces sp*. HU2014 was provided by Henan Institute of Science and Technology in China. Wheat variety of Zhoumai 22 (ZM22) was provided by the Chinese Academy of Agricultural Sciences Qiliying Base in Xinxiang and the HIST Wheat Breeding Center. Green bristlegrass seeds were purchased on line (https://www.lvbad.com/).

Seed pre-germination. Wheat (ZM22) seeds were washed with distilled water, then they were laid out on a tray and covered with gauze. Seeds were kept moist, the water was changed 2~3 times a day. After 24 hours, the exposed seeds were selected for research. Seeds of green bristlegrass were kept in distilled water at 55℃ for 10 min, then they were wrapped with gauze for germination. Seeds were loosed once every 5 hours for air exchange and water was added in the process. The exposed seeds were selected for research after most of them germinated.

The sensitivity index assessment. In the preparatory work, we had separated four fractions (F2, F4, F6, and F8) from the extracellular fermentation of *Streptomyces sp*. HU2014 (Zhu et al., 2022). Fractions of F2 and F4 were dissolved with sterile water and fractions of F6 and F8 were dissolved in ethanol solution (0.4% vv-1), which reached to 1 mg/mL, 5 mg/mL and 10 mg/mL concentration, respectively. Sterile water (named CK) and 0.4% ethanol solution were as controls. Solution of 10 mL per treatment was added on the filter paper which fully covered the inside Petri dish, then 5 per-germinated ZM22 and green bristlegrass seeds were cultured in each Petri dish for 5 days at (25±1)℃, respectively. Every treatment was in triplicates. The shoot and root length of all seedlings in Petri dishes were measured using vernier caliper.

The response index (RI) which measures the treatment responses (T) in relation to their control responses (C) was calculated according the method of Williamson (Williamson et al., 1988). RI is defined as follows: If T < C, then RI = C/T- 1; if T ≥ C, RI = 1-C/T. Negative RI values indicated inhibition of plant growth; positive RI values indicated stimulation of plant growth.

Statistics. Each experiment was done thrice with three biological replicates. Basic data were recorded and processed by Excel (Office 2020). Results were expressed as mean±standard deviation.

Results. In this study, we assessed the allelopathy of four fractions of HU2014 culture on wheat and green bristlegrass.

For wheat, the results showed that four fractions had a certain allelopathic effect on the seedling shoot and root length (Fig. 1, Table 1).

The allelopathy of inhibiting the seedling and root growth was more powerful with the increasing concentration of F2 fraction except for 1 mg/mL. Fraction of F2 at 10 mg/mL had the strongest inhibition on the shoot and root length, the RI values were -0.53 and -0.22, respectively. It was noticed that F2 fraction at 1 mg/mL promoted the shoot length (RI = 0.01). The allelopathy of inhibiting the seedling and root growth was also stronger with the increasing concentration of F4 and F6 fractions. The two fractions at 10 mg/mL had strongest inhibition on the shoot and root length. The RI values for the shoot length were -0.30 and -0.76, and for the root length were -0.58 and -0.62, respectively. Fraction of F8 at 5 mg/mL had the highest inhibition, whereas fraction of F8 at 10 mg/mL had the lowest inhibition on the shoot length (RI were -0.66 and -0.47, respectively). As for root length fraction of F8 at 10 mg/mL had the highest inhibition, whereas F8 at 5 mg/mL had the lowest inhibition (RI were -0.66 and -0.43, respectively).

For green bristlegrass, it was concluded that four fractions had a strong inhibitory effect on the shoot and root length. Moreover, allelopathy of F2 fraction grew with the concentration increasing, while there was no such correlation in relation of other three fractions (Fig. 2, Table 2).

Fraction of F2 at 10 mg/mL had the strongest inhibition on the seedling and root length and the RI values were -0.73 and -1.00, respectively. Fraction of F4 at 5mg/mL had the highest inhibition on the seedling length (RI = -0.69), and at 10 mg/mL had the highest inhibition on the root length (RI = -0.85). Fraction of F6 at 10 mg/mL had the highest inhibition on the shoot length (RI = -0.59), and at 5 mg/mL had the highest inhibition on the root length (RI = -0.80). Fraction of F8 at 5mg/mL had the highest inhibition on the shoot length (RI = -0.47), and at 10 mg/mL had the highest inhibition on the root length (RI = -0.93).

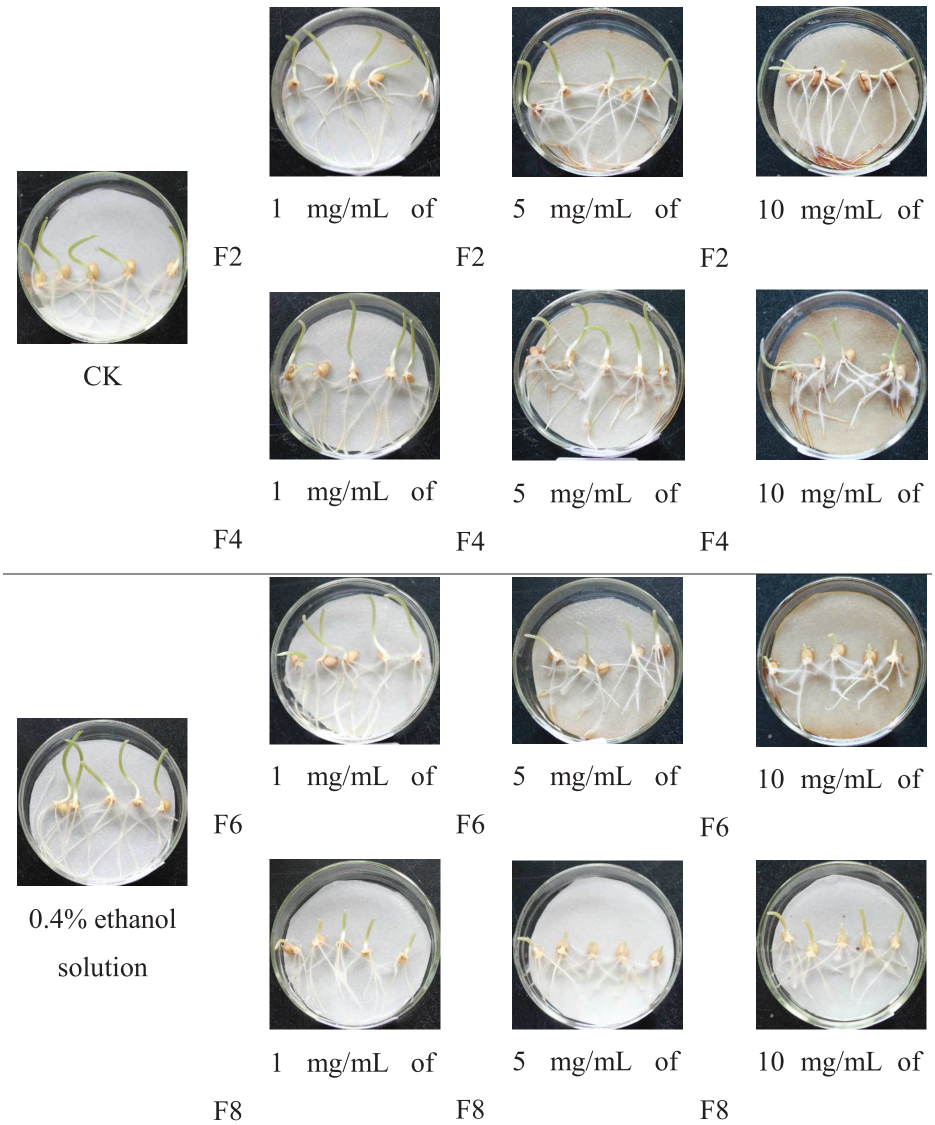

Fig. 1. The seedling growth of wheat after four fractions treatment

Table 1

The sensitivity index of wheat seedling and root length

Concentration, mg/mL		RI value (seeding length)	RI value (root length)
F2	1	0.01±0.45	-0.10±0.99
	5	-0.22±0.72	-0.11±1.09
	10	-0.53±0.32	-0.22±0.55
F4	1	-0.06±0.71	-0.34±0.77
	5	-0.08±0.90	-0.50±0.39
	10	-0.30±0.67	-0.58±0.41
F6	1	-0.02±0.66	-0.34±0.38
	5	-0.45±0.19	-0.54±0.93
	10	-0.76±0.33	-0.62±0.88
F8	1	-0.50±0.67	-0.58±0.97
	5	-0.66±0.58	-0.43±0.59
	10	-0.47±0.97	-0.66±0.43

Note: F2, F4, F6 and F8 represents four ethanol extracts respectively. Each treatment was done three times with three biological replicates. 1, 5, and 10 represent 1mg/mL, 5 mg/mL, and 10 mg/mL, respectively.

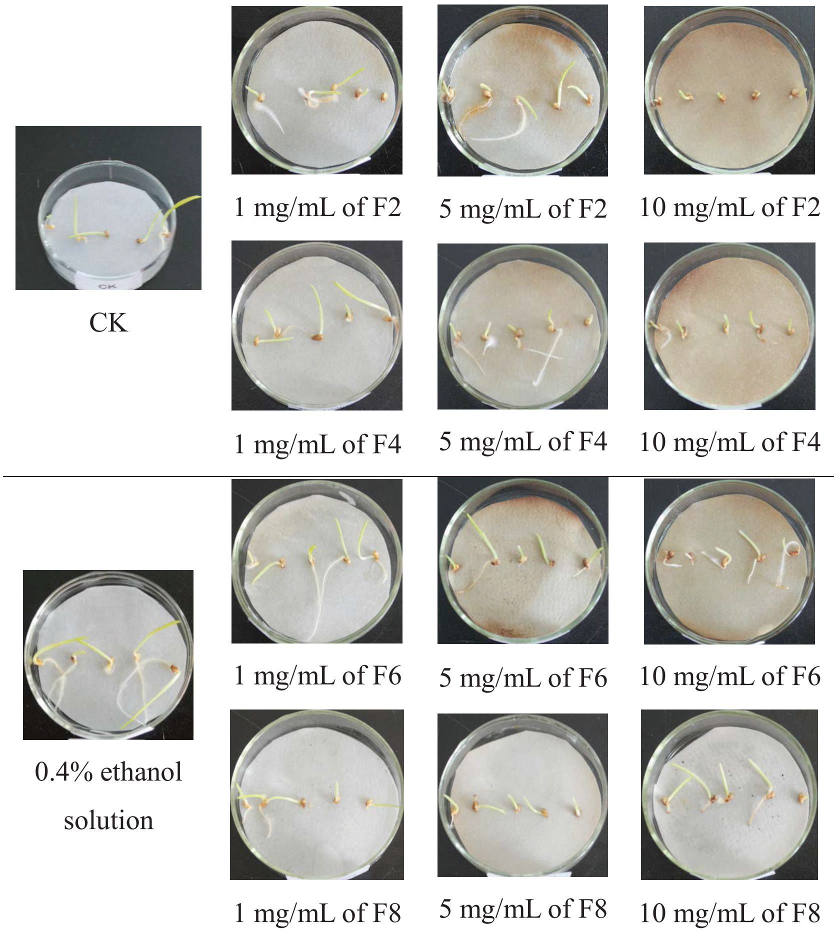

Fig. 2 The seedling growth of green bristlegrasss after four fractions treatment

Table 2

The sensitivity index of green bristlegrass seedling and root length

Concentration, mg/mL		RI value (seedling length)	RI value (root length)
F2	1	-0.13±0.41	-0.08±0.09
	5	-0.35±0.54	-0.53±0.12
	10	-0.73±0.23	-1.00±0.34
F4	1	-0.14±0.61	-0.32±0.66
	5	-0.69±0.33	-0.30±0.65
	10	-0.50±0.41	-0.85±0.73
F6	1	-0.25±0.83	-0.26±0.07
	5	-0.29±0.76	-0.80±0.45
	10	-0.59±0.54	-0.45±0.32
F8	1	-0.40±0.56	-0.83±0.33
	5	-0.47±0.23	-0.93±0.41
	10	-0.37±0.11	-0.82±0.47

Note: F2, F4, F6 and F8 represents four ethanol extracts respectively. Each treatment was done three times with three biological replicates. 1, 5, and 10 represent 1mg/mL, 5 mg/mL, and 10 mg/mL, respectively.

Discussion. Many studies had shown that the symbiotic mechanism of plants and microorganisms is largely related to allelopathy (Muller et al., 2012). The established role of soil microbes in plant health has been consolidated in studies of allelopathy (Mishra et al., 2013). For example, *Ageratina adenophora* invasion promoted an increase of *Bacillus cereus*, which in turn induced a positive feedback effect on *A. adenophor*a (Sun et al., 2021). *Burkholderia* sp. LS-044 is a potential allelochemical-metabolizing bacterium in rice rhizosphere, which got involved in mitigating autotoxicity produced by bacteriostatic-dose of meropenem (Hameed et al., 2019). Simultaneously, the negative allelopathy of microbes on weeds provides an idea for the research and development of new microbial herbicides (Francisco et al., 2019). Some studies reported that soil microorganisms play the beneficial role in weed management (Mishra et al., 2013; Nichols et al., 2015; Xiao et al., 2020). Xi et al. came to a conclusion that *Streptomyces rochei* D74 combined maize rotation could suppress *Orobanche cumama* seed germination (Xi et al., 2022). In our study, the effect of allelochemicals on wheat and green bristlegrass is related to the concentration of allelochemicals and the part of plants. It should be emphasized that low concentration of F2 fraction promoted the wheat seedling growth, but all fractions of HU2014 at different concentration had inhibition on green bristlegrass. This provides a good experimental basis for developing the function of HU2014 to promote growth and inhibit weeds. The allelochemicals separation would be the next work.

Microorganisms have the potential ability to promote crop growth and control weeds in order to make full use of the positive effects of allelochemicals in the agricultural ecosystem. Developing of natural pesticides and growth regulators have important theoretical value and practical significance for the effective use of resources, safe environment, and the development of sustainable agriculture. However, the activity of microorganisms in the environment may differ substantially from laboratory observations (Vurukonda et al., 2018). The research about the application of HU2014 in farming environment will be continued.

Conclusions. In this study, we assessed the allelopathy of HU2014 metabolites on wheat and green bristlegrass. Four fractions of the cell-free filtrates of HU2014 culture broth had a certain inhibitory effect on the shoot and root length of wheat and bristlegrass except fraction of F2: at 1 mg/ml it promoted the wheat shoot length. These results provide new evidence that HU2014 has allelopathy on plant. Concurrently, the metabolite of this strain can promote crop growth but inhibit weed growth at low concentration. Therefore, HU2014 would be a promising agent as biofertilizer and herbicide.

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Алелопатична дія метаболітів, продукуваних Streptomyces sp. HU2014, на пшеницю та мишій зелений

Ризобактерії, які стимулюють ріст рослин, широко поширені в ризосфері рослин. Вони забезпечують живлення та продукують деякі антибіотичні речовини для пригнічення фітопатогенів і сприяння росту рослин. Тому вивчення алелопатії є дуже важливою частиною взаємодії між ризобактеріями та рослиною. Streptomyces, рід актиноміцетів, добре відомий своїми біоактивними метаболітами, головним чином – антибіотиками, гормонами та гідролазою, які можуть впливати на ріст рослин. У цьому дослідженні було оцінено алелопатію метаболітів штаму Streptomyces sp. HU2014 на пшеницю та мишій зелений з використанням індексу відповіді RI. Негативні значення RI вказували на пригнічення росту рослин; позитивні показники свідчили про стимуляцію їх росту. Чотири фракції (F2, F4, F6 та F8) з безклітинних фільтратів культурального бульйону HU2014 мали певний вплив на довжину проростків та коренів рослин. Для пшениці результати показали, що фракція F2 при 10 мг/мл мала найбільш виразний ефект пригнічення довжини проростків (RI=-0,53) і кореня (RI=-0,22). Однак фракція F2 при 1 мг/мл сприяла збільшенню довжини проростка (RI=0,01). Фракції F4 і F6 при 10 мг/мл мали найбільше пригнічення довжини проростків та коренів. Для фракції F8 зафіксовано найбільше зменшення довжини проростків (RI = -0,66) при 5 мг/мл і довжини кореня (RI = -0,66) при 10 мг/мл. Для мишію зеленого фракція F2 при 10 мг/мл пригнічувала як довжини проростків (RI = -0,73), так і кореня (RI =-1,00). Фракція F4 показала найвище пригнічення проростку (RI = -0,69) при 5 мг/мл і кореня (RI = -0,85) при 10 мг/мл. Фракція F6 мала найвище пригнічення довжини проростка (RI = -0,59) при 10 мг/мл і кореня (RI = -0,80) при 5 мг/мл. Фракція F8 показала найвищий рівень інгібування довжини проростка (RI = -0,47) при 5 мг/мл і кореня (RI = -0,93) при 10 мг/мл. З наведених вище результатів ми можемо зробити висновок, що чотири фракції мали алелопатичний вплив на довжину проростків і коренів двох досліджуваних видів рослин, за винятком фракції F2 при 1 мг/мл, яка сприяла збільшенню довжини пагонів пшениці. Таким чином, на ранній стадії росту рослин низька концентрація алелопатичних речовин, які продукує HU2014, може сприяти росту пшениці, тоді як ці речовини пригнічують ріст мишію зеленого. Тому цей штам може бути перспективним як у якості біофунгіциду, так і – біогербіциду.

Ключові слова: *Streptomyces, алелопатія, індекс відповіді, пшениця, мишій зелений.*