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Effects of aqueous extract of soil-like substrate made from three different materials on seed germination and seedling growth of rice

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ABSTRACT

Biologically processing rice and wheat straws into soil-like substrate (SLS) and then reusing them in plant cultivation system to achieve waste recycle is very crucially important in Bioregenerative life support system (BLSS). However, rice is a plant with strong allelopathic potential. It is not clear yet that what kinds of raw materials can be processed into proper SLS to grow rice in BLSS. Therefore, in this study, the aqueous extract of SLS made from three different materials including rice straw, wheat straw and rice–wheat straw mixture was utilized to investigate its effects on the seed germination and seedling growth of rice. The gradients of the extract concentrations (soil:water) were 1:3, 1:5, 1:9, and 1:15 with deionized water used as control. The effects of different types of SLS on seed germination and seedling vitality of rice were confirmed by analyzing the germination rate, seedling length, root length, the fresh weight and other indicants. In addition, based on the analysis towards pH, organic matter composition and other factors of the SLS as well as the chlorophyll, hormone content of rice, and the mechanism of the inhibition was speculated in order to explore the preventive methods of the phenomenon. Finally, the feasibility of cultivating rice on SLSs made from the raw materials mentioned above was evaluated and wheat raw was determined as the most appropriate material for growing rice.

1. Introduction

With the development of manned spacecraft engineering, Bioregenerative life support systems (BLSS) with highly closed intra-system mass exchange hold much promise for long-term human life support in Lunar or Mars bases. As a photosynthesizing component in BLSS, higher plants which can not only provide food for human but also reproduce air and water, have been demonstrated to possess good prospects, and have been approved by the researchers in the field of Bioregenerative life support technology from many countries. However, a crucial problem to achieve the intra-system circulation of BLSS is how to utilize the inedible part of the plant [1]. At present, most life support systems employ wheat and rice as their higher plants because they are major crops in many countries according with their diet custom [2]. However, wheat and rice possess a large amount of inedible parts [3,4].

In recent years, the Institute of Biophysics in Russia (Russian Academy of Sciences, Siberian Branch) has developed the procedure of producing a soil-like substrate (SLS) from plant wastes as a new processing technique [5,6]. After

Abbreviations: SLS, soil-like substrate; BLSS, Bioregenerative life support system; IAA, 3-indolylacetic acid; GA, gibberellic acid; ABA, abscisic acid; CTK, cytokinin

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pretreatment, the inedible biomass of the plants is inoculated with oyster mushroom spawn grown on the wheat grains under sterile conditions. When mushroom growth completes, the spent mushroom compost can be transferred from plastic bags to boxes and converted to SLS by earthworms. Finally, the achieved SLS could be used as a substrate for growing plants [7]. By means of this process, the reuse of the inedible parts of the plants within a closed system can be achieved [8]. The feasibility of introducing SLS into closed systems has been proved by experiments. Gros et al. have demonstrated that the SLS made from wheat straw is viable to grow several crops including wheat, beans, cucumber and radish [9,10]. The results showed that their yields when growing on a mature SLS are comparable to the yields when growing on a neutral substrate (expanded clay aggregate) under hydroponics [9,11]. It has been proved that SLS plays a critical role by serving as substrate in the life support system for sustainable utilization of the inedible parts of plants and then, improved the system's air tightness [12,13]. In previous studies, researchers in the laboratory have successfully produced SLS by the straw of rice and wheat, and optimized the SLS production technological process. The results showed that SLS made from straw of rice and wheat is viable to grow lettuce [7,14].

However, some research showed that the straw of rice and wheat released allelochemicals into surrounding environment thus affecting the growth of other plants, and even rice and wheat by released autotoxic substance [15–17]. In the allelopathic test, excessive pH and osmotic potential in the aqueous extract of SLS are found to be the main reasons for the inhibition of seed germination, even the growth of root and stem [18]. Whether the SLS made from straws will have an allelopathic effect on the growth and development of crops is a crucial problem which has not been reported yet.

In this study, upland rice (*Oryza sativa*) was grown with aqueous extract of SLS made from three different materials: rice straw, wheat straw and rice–wheat straw mixture to investigate their effects on seed germination and seedling growth of rice, to evaluate the feasibility of cultivating, and to determine the optimal raw materials to produce SLS to grow rice. The allelopathic effect was mainly measured by biological testing and analysis [19].

2. Materials and methods

2.1. Materials

The upland rice (*O. sativa*) and spring wheat (*Triticum aestivum*) obtained from the experimental fields of the Chinese Academy of Agricultural Sciences (CAAS) were chosen as the raw materials to produce SLS [2]. All the SLSs used in this experiment were produced and measured according the methods by Chengying and Hong, and the duration of the process of the straw bioconvertion (SLS production) was 93 days [7]. The upland rice (*O. sativa*) was also used for seed germination and seedling growth.

2.2. Preparation of aqueous extract of SLS

In this study, aqueous extract of SLS was obtained by mixing the SLS with deionized water at the ratio of 1 g:2 ml (soil:water) oscillating for 1 h, centrifuging for 10 min with 4000 rmp, and then extracting the upper liquid by filtration for 24 h at 30 °C. The aqueous extract was diluted with deionized water; the gradients of the extract concentrations (soil:water) were 1:3, 1:5, 1:9; and 1:15. Deionized water was used as control. The characteristics of the aqueous extract of SLS as shown in Table 1.

2.3. Germination and seedling planting experiment

The seeds were germinated in 15-cm Petri dishes with 10 ml aqueous extract of SLS or deionized water. Each dish contained 100 seeds, and each group has three duplications. All the dishes were controlled in a thermostat incubator at 25 °C, with 2 ml supplementary aqueous extract added to each plate every day. Germination was measured 7 days after seeds sowing.

After germination, the seedlings planting experiment was namely established. The temperature was controlled at 23–28 °C, and relative humidity at 50–70%. The light intensity was controlled within the range of 200 l mol $m^{-2} s^{-1}$ photosynthetic photon flux densities by continuous lighting of fluorescent tubes. The seedlings were watered 1–2 times per day during the growth process. Ten seedlings were chosen from each dish for determinations of root length, shoot length, and total fresh weight at the 14th day.

2.4. Determination of chlorophyll content in seedlings

Chlorophyll was determined by the method published by Harris [20]. Chlorophyll of leaf segments was extracted into 95% ethanol and the absorbance (A) was measured at 665 nm and 649 nm on a spectrophotometer. The chlorophyll content was calculated by the following equations:

$$\text{Total chlorophyll (chl t)} = \text{chlorophyll a(chl a)} + \text{chlorophyll b (chl b)} = 6.27\,(A665) + 18.08\,(A649)$$

$$\text{Total chlorophyll content} = \text{chl t} \times V \times N/W$$

where A665 is the absorbance (A) at 665 nm, A649 is the absorbance (A) at 649 nm, V is the volume of the

Table 1
Characteristic of SLS made from three different materials.

	pH				Organic matter (%)	Total N (g/kg)	Total P (g/kg)	Total K (g/kg)
	1:3	1:5	1:9	1:15				
RSLS	7.59	7.47	7.23	7.12	16.07	4.13	7.38	39.40
RWSLS	7.73	7.51	7.46	7.35	14.38	5.16	6.54	35.71
WSLS	7.3	7.21	7.11	7.05	17.53	6.58	6.21	31.32