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Growth Inhibitory Activity of Tea-Seed Saponins and Glyphosate to Weed Seedlings

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Abstract

It was proved that tea-seed saponins (TSS) showed the growth inhibitory activity to the weed seedlings, early watergrass (*Panicum Crus-galli L.*), green foxtail (*Setaria viridis Beauv. L.*) and white clover (*Trifolium repens L.*) at concentrations more than 10 mg/L. However, this activity was lower than agricultural chemicals. The additive growth inhibitory effect of TSS and glyphosate (isopropylammonium N-(phosphonomethyl)glycinate), which is one of the most popular weed killers in Japan, was investigated to prove that TSS additively increased the growth inhibitory effect of glyphosate to early watergrass seedlings. This result suggests that TSS could be utilized as growth inhibitory agents of weed seedlings in combination with other agricultural chemicals.

Discipline: Floricultural science
Additional key words: agricultural chemical, emulsifying agent

Introduction

Saponins are frequently present in certain plants as a complex mixture and they are often detected in plant extract via their hemolytic activities or on the basis of their foam producing properties[2]. It has been recognized that tea-seed saponins (TSS) account for more than 10% per dry weight of the tea seed[1], and that they display various physiological functions such as anti-expectorant[7], anti-inflammatory properties[6] and suppression effect on alcohol absorption[9] etc. Recently we have reported the development of a simple preparation method for TSS and their physiological activities such as antiyeast activity[10], a growth inhibitory effect on salt tolerant yeast (*Zygosaccharomyces rouxii*) in the presence of sodium chloride[8], as well as a control effect against insect pests and mites[4].

Until now, TSS had not been efficiently utilized in spite of their interesting biological activities, because their activities were found to be lower in comparison with those of other chemical compounds. Nevertheless, TSS could be utilized as are other natural products as biological agents since they are less polluting to the environment than their synthetic chemical counterparts. The oral toxicity of TSS has been estimated to be low by the oral repeated administration toxicity study in rats[3].

Only a few papers on the growth regulating activity of saponins to plants have been presented so far. Mayevsky and Marchaim found that the immersion of cotton seeds in aqueous lucerne saponin led to the inhibition of germination because of the respiratory inhibition of cotton seeds[5]. Wickremasinghe et al. reported the effect of ethephon (2-chloroethylphosphonic acid) in combination with TSS or tea root saponins and suggested the increase of root weight and root length of tea in the nursery and the field[11]. However, not only was the effect insignificant, but also an independent effect of saponins was not evident.

In this report, we investigated the growth inhibitory activity of TSS to weed seedlings. We also referred to an efficient utilization of TSS as growth inhibitory agents.

Materials and Methods

TSS were prepared according to the method reported by Yamauchi et al.[10] from tea seeds of *cv.* Yabukita which were collected in a tea field of NIVTS (Kanaya Tea Research Station).

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Water for this experiment was prepared by distillation and deionization (Milli-Q, Millipore). Glyphosate was purchased from Kanto Chemical Co., Ltd. (Tokyo, Japan). All chemical reagents were of special-reagent grade. White clover seeds were purchased from Takii Seed Co. (Kyoto, Japan). Weed seeds (early watergrass and green foxtail) were provided by Bayer Crop Science Co., Ltd. (Tokyo, Japan).

1. Growth inhibitory activity of TSS and glyphosate to weed seedlings

Thirty weed seeds were placed on two sheets of filter paper moistened with TSS solutions at the concentrations of 0 (control), 10, 25, 50, 100 and 250 mg/L or glyphosate solutions at the concentrations of 0 (control), 5, 10 and 25 mg/L in 9-cm petri dishes, which were incubated at 25ºC with a daily 12 h photo period of 2,500 lux. The lengths of the hypocotyl and seminal root of the seedlings were measured 4–5 days after incubation. Each treatment consisted of three replications.

2. Additive growth inhibitory effect of TSS and glyphosate to early watergrass seedlings

Thirty early watergrass seeds were placed on two sheets of filter paper moistened with a mixture of TSS (0, 5, 10, 25 and 50 mg/L) and glyphosate (1.0, 2.5 and 5.0 mg/L) solution in 9-cm petri dishes, which were incubated at 25ºC with a daily 12 h photo period of 2,500 lux. The lengths of the hypocotyl and seminal root of the seedlings were measured 4–5 days after incubation. Each treatment consisted of three replications.

Results and Discussions

1. Growth inhibitory activity of TSS and glyphosate to weed seedlings

Early watergrass, green foxtail and white clover were selected in this study because they have been often used to evaluate the inhibitory activity of agricultural chemicals and have an almost 100% germination rate. As shown in Table 1, TSS inhibited the growth of the hypocotyl at above 100 mg/L for early watergrass, 50 mg/L in green foxtail and 10 mg/L for white clover, respectively. On the other hand, the growth of the seminal root was inhibited at above 25 mg/L for early watergrass and 50 mg/L for green foxtail and white clover. This activity was different depending on the species and parts of the weed seedlings. These results suggested that TSS might show growth inhibitory activity to weed seedlings at concentrations higher than 10 mg/L. The germination rate of all weed seeds was almost 100%, meaning that TSS did not affect the germination of the weed seeds used in this study.

Glyphosate, known as a weed killer which inhibits the synthesis of amino acids, was selected as a representative agricultural chemical, because it is one of the most popular weed killers in Japan and is easily soluble in water without any spreading agents, which is convenient for the preparation of test solutions. Table 2 shows the growth inhibitory activity of glyphosate to early watergrass and green foxtail seedlings. Glyphosate inhibited the growth of the hypocotyl and seminal root at 25 mg/L and 10 mg/L for early watergrass. On the other hand, the growth of the hypocotyl and seminal root was inhibited at

Table 1. Growth inhibitory activity of TSS to weed seedlings

Seedlings	TSS (mg/L)					
	0 (control)	10	25	50	100	250
Early watergrass						
Hypocotyl (cm)	2.36 ± 0.76 a	2.25 ± 0.74 a	2.09 ± 0.69 a	2.11 ± 0.59 a	1.97 ± 0.57 b*	1.57 ± 0.61 c**
Seminal root (cm)	3.21 ± 0.92 a	3.20 ± 0.91 a	2.67 ± 0.67 b**	1.20 ± 0.38 c**	0.62 ± 0.25 d**	0.01 ± 0.00 e**
Green foxtail						
Hypocotyl (cm)	1.83 ± 0.25 a	1.73 ± 0.27 a	1.85 ± 0.22 a	1.68 ± 0.29 b**	1.50 ± 0.32 c**	0.82 ± 0.26 d**
Seminal root (cm)	2.85 ± 0.46 a	2.74 ± 0.55 a	2.79 ± 0.53 a	1.53 ± 0.41 b**	0.61 ± 0.23 c**	0.25 ± 0.07 d**
White clover						
Hypocotyl (cm)	0.63 ± 0.07 a	0.56 ± 0.06 b*	0.54 ± 0.05 b	0.35 ± 0.05 c**	0.28 ± 0.04 d**	0.20 ± 0.02 e**
Seminal root (cm)	2.08 ± 0.28 a	2.06 ± 0.30 a	2.04 ± 0.27 a	1.67 ± 0.20 b**	1.19 ± 0.19 c**	0.54 ± 0.05 d**

Data represent the means ± SD of three replicated values.
Different letters within a line indicate significant differences, *: $p<0.05$, **: $p<0.01$.
A statistical analysis was performed by a Bonferroni multiple comparison method.

10 mg/mL for green foxtail. The activity of glyphosate was 2.5–5 times more intensive than that of TSS. The germination rate was almost 100%, meaning that glyphosate did not affect the germination of the weed seeds.

2. Additive growth inhibitory effect of TSS and glyphosate to early watergrass seedlings

Since the growth inhibitory activity of TSS was lower than glyphosate as mentioned above, the additive inhibitory effect of TSS and glyphosate was investigated to develop an efficient utilization of TSS as growth inhibitory agents. As shown in Table 3, the growth of the hypocotyl in early watergrass was inhibited by TSS at 50 mg/L in combination with glyphosate at 2.5 mg/L. Individual concentrations of TSS and glyphosate in the growth inhibition of the hypocotyl were 100 and 25 mg/L, respectively. The concentration of effective growth inhibition was reduced to about 1/2 for TSS and 1/10 for glyphosate. The growth of the seminal root in early watergrass was inhibited by TSS at 25 mg/L in combination with glyphosate at 1.0 mg/L. Individual concentrations of TSS and glyphosate in the growth inhibition of the seminal root were 25 and 10 mg/L, respectively. The concentration of effective growth inhibition was not reduced for TSS, but reduced to about 1/10 for glyphosate. These results suggest that TSS additively enhanced

Table 2. Growth inhibitory activity of glyphosate to early watergrass and green foxtail seedlings

Seedlings	Glyphosate (mg/L)			
	0 (control)	5	10	25
Early watergrass				
Hypocotyl (cm)	2.61 ± 0.63 a	2.63 ± 0.55 a	2.63 ± 0.72 a	2.12 ± 0.65 b**
Seminal root (cm)	3.68 ± 0.70 a	3.18 ± 0.55 a	2.43 ± 0.39 b**	1.62 ± 0.24 c**
Green foxtail				
Hypocotyl (cm)	2.09 ± 0.44 a	2.16 ± 0.36 a	1.93 ± 0.39 b**	1.82 ± 0.41 c*
Seminal root (cm)	3.12 ± 0.76 a	3.13 ± 0.76 a	2.25 ± 0.57 b**	1.18 ± 0.31 c**

Data represent the means ± SD of three replicated values.
Different letters within a line indicate significant differences, *: $p<0.05$, **: $p<0.01$.
A statistical analysis was performed by a Bonferroni multiple comparison method.

Table 3. Additive effect of TSS on the growth inhibition in early watergrass seedlings in combination with glyphosate

TSS (mg/L)	Glyphosate (mg/L)		
	1.0	2.5	5.0
	Hypocotyl (cm)		
0	2.42 ± 0.76 a	2.32 ± 0.67 a	2.64 ± 0.67 a
5	2.36 ± 0.69 a	2.32 ± 0.56 a	2.64 ± 0.75 a
10	2.26 ± 0.76 a	2.23 ± 0.58 a	2.63 ± 0.68 a
25	2.20 ± 0.55 a	2.23 ± 0.52 a	2.26 ± 0.72 b**
50	2.22 ± 0.62 a	1.95 ± 0.62 b**	2.23 ± 0.59 b
	Seminal root (cm)		
0	3.37 ± 0.88 a	3.27 ± 0.69 a	3.01 ± 0.59 a
5	3.36 ± 0.82 a	3.17 ± 0.56 a	2.94 ± 0.61 a
10	3.27 ± 1.06 a	3.14 ± 0.69 a	2.93 ± 0.61 a
25	2.71 ± 0.65 b**	2.64 ± 0.58 b**	2.45 ± 0.46 b**
50	1.21 ± 0.42 c**	1.20 ± 0.38 c**	1.13 ± 0.44 c**

Data represent the means ± SD of three replicated values.
Different letters within a row indicate significant differences, *: $p<0.05$, **: $p<0.01$.
A statistical analysis was performed by a Bonferroni multiple comparison method.

the growth inhibitory effect of glyphosate and the amount of glyphosate used could be reduced. Studies on the mechanism of the additive inhibitory effect are in progress.

TSS are formally recognized as emulsifying agents in food additives based on their surface activities. At the same time, TSS show a growth inhibitory activity to weed seedlings and exert additive growth inhibitory activity to weeds in combination with other agricultural chemicals. In consideration of these characteristics, it would be most practical to utilize TSS as spreading agents of agricultural chemicals, for example weed killers.

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