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Effects of Herbicides on the Growth and Tannin Accumulation in a Medicinal Plant, *Geranium thunbergii* SIEB. *et* ZUCC.

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SYNOPSIS

Herbicide influences greatly the many sites of metabolic processes in the plants. In this study, we examined their effects on the growth and tannin accumulation in *Geranium thunbergii* at a weak sub-lethal concentration. The herbicide was applied 2,4-D at 10^{-5}, 10^{-6}, 10^{-7} M, glyphosate at 10^{-4}, 10^{-5}, 10^{-6} M, and propham (IPC) at 10^{-3}, 10^{-4}, 10^{-5} M. Foliage spraying was conducted one month before harvest. Any herbicides at these dosages did not reduce the dry weight of aerial parts. The increase in the tannin concentration in leaf blades was obtained by 10^{-6} M 2,4-D. On the other hand, propham at 10^{-3} M decreased the tannin concentration. The tannin in stems was little affected by these herbicide treatments. The results of this study suggested that some herbicides at a given concentration affect the secondary metabolism of *Geranium thunbergii* plant.

INTRODUCTION

There are many reports on the effect of herbicides on the secondary compounds. Some herbicides reduced the secondary compounds and other ones increased them. The effects of herbicides are not so clear-cut because the type and mechanism of damage varies considerably among herbicides and also the effectiveness is different among species. The concentrations or methods of herbicides depend on the plant species. The effects of herbicides on the secondary metabolism in the plants have not been studied in detail, which is why it is difficult to understand the relation between the primary site of action and their effects. Devine *et al.*[1] reported that the concentration of tannins derived from benzoic acid is increased by glyphosate. Thus, this study was conducted to clarify the effects of some herbicides on growth and tannin accumulation in a medicinal plant, *Geranium thunbergii*.

MATERIALS AND METHODS

Plant material and cultivation *Geranium thunbergii* plants were grown by the same method as mentioned in our previous paper, were used in this study. In January 1992, these two plants were transplanted into a rectangular plastic pot 15 cm wide, 32 cm long, and 14 cm deep, filled with a mixture of vermiculite and sandy loam soil (1 : 1 ; v/v). All plants were grown in a plastic greenhouse and watered twice or three times a week. As chemical fertilizers, 1.6 g $NH_4(SO_4)_2$,

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1.4 g $CaH_4(PO_4)_2$, and 0.7 g K_2SO_4, were applied to each pot. The experiment was conducted from January to October, 1992. The data were collected twice a year in July and October, the first and second cropping, respectively. The pots were randomly divided into 2 groups, one of them were used for the first experiment. In the other pots, plants were cut with aerial parts in July, with a remaining basal stem of approximately 2 to 3 cm to allow them to grow new shoots from July to October, and were used for the second experiment.

Experimental procedures Plants were sprayed with three kinds of herbicides by three levels of concentration at 10^{-5}, 10^{-6}, or 10^{-7} M 2,4-D, 10^{-4}, 10^{-5}, or 10^{-6} M glyphosate, and 10^{-3}, 10^{-4}, or 10^{-5} M propham. The concentrations of herbicide in this study were determined at the lower damaged to the mortality of *Raphanus sp.* seedlings in our prior observation. To all sprayed treatments 0.1% Tween 20 surfactant was added. These herbicides were sprayed to the foliage one month before harvest ; in June for the first cropping and in September for the second one, respectively. The application was done by a hand sprayer with the total volume of 50 ml per plant which was sprayed to run off on the whole plant in the evening application. Control plants were sprayed with clean water.

Measurement of plant growth At harvest time, the plants were separated into leaves, stems, and roots. These were dried at 40°C for 72 h and weighed for their dry weights.

Assay of tannin concentration Dried leaf blades of the third and fourth leaves from the apex and whole stems were finely grounded. These samples were weighed 150 mg and 500 mg for leaves and stems, respectively. The samples were extracted with 10 ml 60% acetone. The amount of tannin in extracts was determined by the colorimetric method of Okuda and co-workers[2]. The tannin concentration was expressed as a percentage of the original dry weight.

Statistical analysis Results were given as the means of three repeated experiments. Statistical analysis was based on a completely randomized experimental design using a Stat Flex statistical analysis package (View Flex Company, Tokyo[3]).

RESULTS

Table 1. shows the dry weights of the plants sprayed with herbicides in the first cropping. In

Table 1. Dry weights of the plants sprayed with herbicides one month before harvest in the first cropping

Treatment	conc. (M)	Aerial parts (g)	Leaves (g)	Stems (g)	Roots (g)
Control		10.44±0.25 ns	3.31±0.30 ns	6.36±0.29 abcde	6.65±0.87 bcde
2,4-D	10^{-5} M	11.99±3.03	3.61±0.83	7.32±2.05 abcd	8.85±1.68 a
2,4-D	10^{-6} M	13.71±2.02	4.64±0.64	7.85±1.49 ab	8.16±1.36 abc
2,4-D	10^{-7} M	9.28±1.32	3.39±0.64	5.04±1.03 bcde	7.22±0.47 abcde
Glyphosate	10^{-4} M	11.80±2.92	4.13±1.46	6.38±1.05 abcde	6.48±0.53 bcde
Glyphosate	10^{-5} M	12.35±1.10	3.97±0.56	7.38±0.62 abc	8.19±0.38 ab
Glyphosate	10^{-6} M	14.29±5.26	4.03±1.37	9.09±3.34 a	7.41±0.31 abcde
Propham	10^{-3} M	7.20±1.52	2.45±0.39	3.93±0.81 e	5.92±1.00 e
Propham	10^{-4} M	10.61±2.28	3.13±0.74	6.71±1.71 abcde	6.62±0.57 bcde
Propham	10^{-5} M	11.25±1.65	3.64±0.62	6.83±1.14 abcde	8.10±0.85 abcd

Values are means ± SD of three replicates. Means with different letters in the same column show significant differences by Duncan's multiple range test at $p=0.05$. ns, not significant.

general, the dry weights of aerial parts and leaves did not significantly differ among herbicide applications. Stem and root dry weights of the plants sprayed with 10^{-3} M propham were significantly reduced. Also, the number of leaves, and stem length (data not shown) were decreased by 10^{-3} M propham. The plants sprayed with 10^{-5} M 2,4-D had greater root weight than the control plants.

Tannin concentration in leaf blades of the first cropping was little affected by any treatments although the concentration tended to decrease in the propham treatments (Fig. 1A). The tannin concentration at 10^{-3} M propham was 25% less than that of control, only 10^{-6} M 2,4-D showed a little increase in the tannin concentration. The concentration in stems of the plants sprayed with the herbicides was not different from that of control (Fig. 2A).

Table 2. shows the dry weights of plants sprayed with herbicides in the second cropping. Herbicide applications in this experiment did not affect the dry weight. The tannin concentration in leaf blades of the plants was reduced only with 10^{-3} M propham spraying (Fig. 1B). The

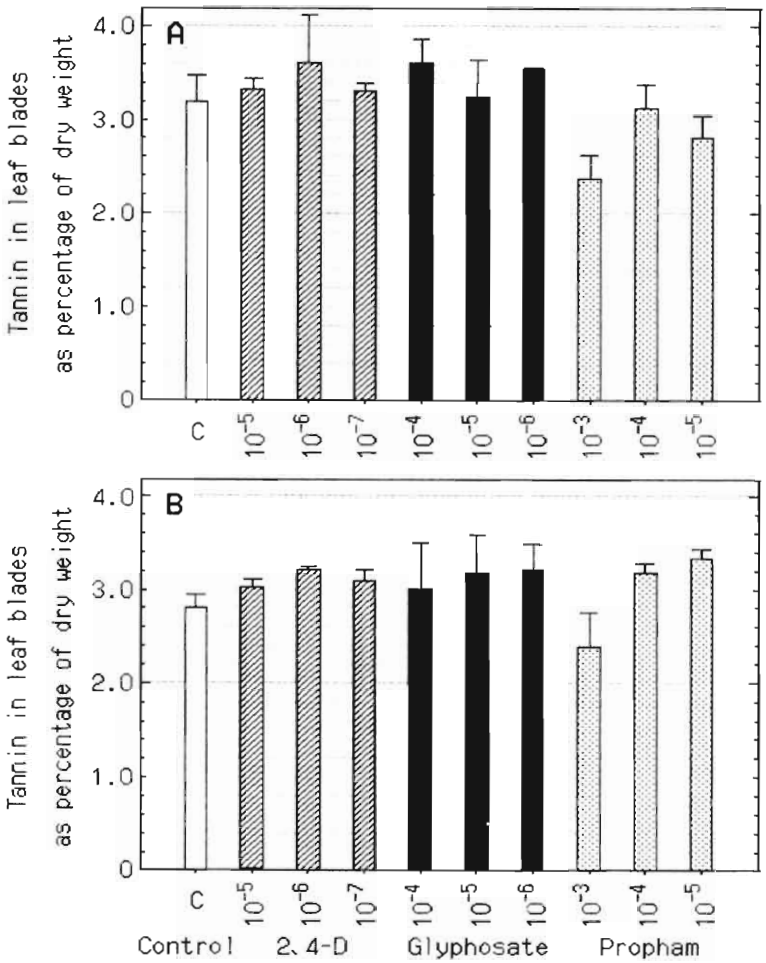

Fig. 1 Tannin concentration in leaf blades of the first (A), and second (B) cropping after spraying herbicides one month before harvest
Bars represent means ± SD of three replicates.

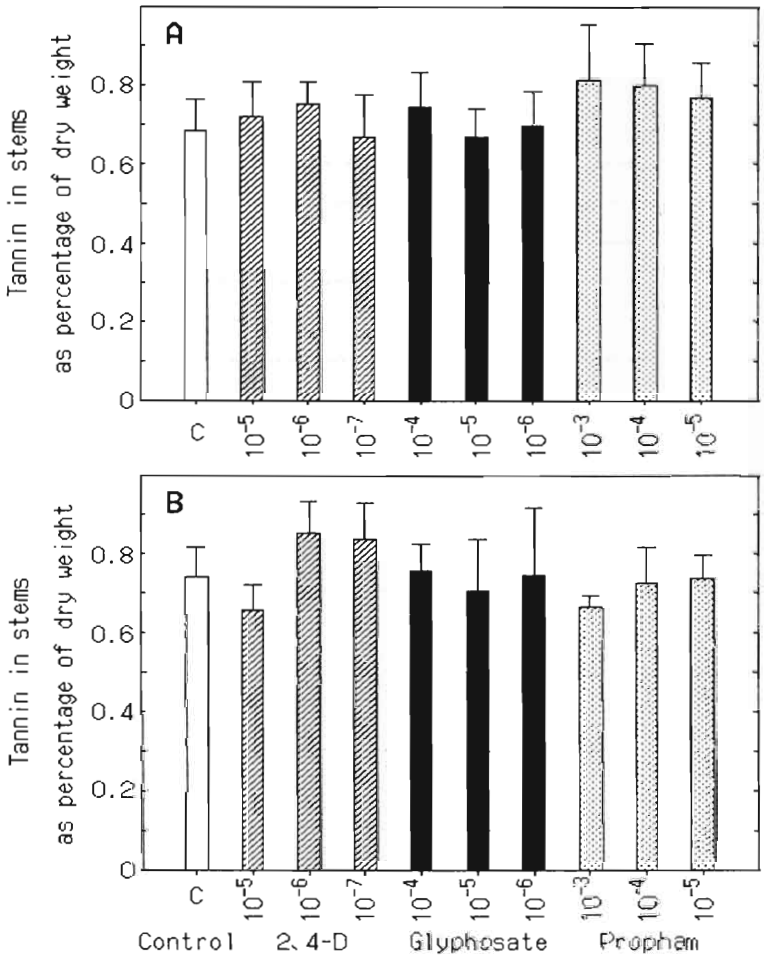

Fig. 2 Tannin concentration in stems of the first (A), and second (B) cropping after spraying herbicides one month before harvest.
For details, see the legend of Fig. 1.

Table 2. Dry weights of the plants sprayed with herbicides one month before harvest in the second cropping

Treatment	conc. (M)	Aerial parts (g)	Leaves (g)	Stems (g)	Roots (g)
Control		11.48±0.30 ns	3.43±0.81 ns	5.06±0.20 ns	8.37±1.98 ns
2,4-D	10^{-5} M	9.31±3.66	2.58±1.09	4.15±1.40	6.83±1.21
2,4-D	10^{-6} M	8.06±1.75	2.35±0.85	3.77±0.73	6.23±0.70
2,4-D	10^{-7} M	11.15±1.98	3.24±1.00	5.32±1.09	7.34±1.06
Glyphosate	10^{-4} M	8.25±2.26	2.47±0.84	3.76±0.89	6.92±0.95
Glyphosate	10^{-5} M	11.86±4.31	3.12±1.60	4.73±1.98	7.22±1.00
Glyphosate	10^{-6} M	9.52±1.62	3.00±0.78	3.86±0.90	8.02±2.40
Propham	10^{-3} M	10.55±1.28	4.66±1.13	4.08±0.68	6.46±0.42
Propham	10^{-4} M	11.17±1.29	3.23±0.99	4.78±0.96	7.07±0.69
Propham	10^{-5} M	9.77±0.98	2.75±0.81	4.13±0.20	8.95±2.08

Values are means ± SD of three replicates. ns, not significant.

concentration was 14% less than that of control. On the other hand, the increase of the tannin concentration in leaf blades was 10-14% by 2,4-D at 10^{-6} M and 10^{-7} M compared with the control. Any other treatments did not affect the tannin concentration. Also, the tannin concentration in stems was not affected by herbicide applications (Fig. 2B).

DISCUSSION

In our studies, any herbicides did not reduce the dry matter production of *Geranium thunbergii*. In either experiments of two croppings any herbicides except 10^{-3} M propham did not affect the plant growth. Propham at 10^{-3} M decreased the stem and root dry weights in the first cropping. This results indicated that propham at high concentration inhibits the metabolic processes of this herbal crop. However, the damage was not observed in the second cropping. Plant age or season at application may be influence the adverse effectiveness of propham.

The tannin concentration in leaf blades was increased by 10^{-6} M 2,4-D in this experiment. The influence of exogenous application of 2,4-D may be related to endogenous-auxin change. Lee *et al.*[4] and Grambow and Langenbeck-Schwich[5] indicated that many phenolic compounds affect the rate of enzymatic oxidation of indole-3-acetic acid (IAA). However, our findings did not show the increase of tannin concentration by the glyphosate applications. Lydon and Duke[6] found that the concentration of gallic acid is markedly increased by glyphosate in certain tissues of pigweed and soybean. The conflicting results with different species and system could be due to glyphosate causing different qualitative changes in phenolic compounds in the species studied.[1] The response of plants in tannin concentration in leaf blades to propham application differed with the crop, and developmental stage.

In conclusion, the herbicide applications used in this experiment did not affect the dry weight of aerial parts of *Geranium thunbergii* plant. Spraying with 10^{-6} M 2,4-D increased the tannin concentration in leaf blades. The mild to weak stress caused by herbicide influenced the synthesis of change a secondary metabolite "tannin". These findings suggested that hormonal balance affects by herbicides play a role in the secondary metabolite in *Geranium thunbergii* plant.

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除草剤が薬用植物，ゲンノショウコ，の成育とタンニン生産に及ぼす影響

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要 約

除草剤は本来防除対象植物の殺草または成育の抑制を目的として使用されるものであるが，他方薬剤によりそれぞれ植物代謝過程の異なった部分に特異的阻害作用をもつことが注目されている。

本報告では，日本において薬草として広く利用されてきたゲンノショウコに対して，3種類の作用機作の異なる除草剤，2,4-D，ラウンドアップ（glyphosate）およびIPC（propham）を用い，それぞれ大根の芽生えについてあらかじめ検定した致死限界量以下の3段階の薬剤濃度で，収穫1か月前に葉面散布をした。

この結果，3種の除草剤は，いずれも本植物の乾重生産量を低下させなかったが，10^{-6} M 2,4-Dは葉のタンニン含量を増加させたが，10^{-3} M IPCの場合，葉のタンニン含量は1期作（1月-7月）ではやや減少し，2期作（7月-10月）では低濃度区でやや増加させた。

これらの結果は，除草剤によってはゲンノショウコの成育を阻害しない濃度で，その2次代謝物質の含量に影響を与えることを示唆するものであろう。