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Effects of Application Levels of N-P-K Fertilizer on the Growth and Yield of a Medicinal Plant, *Geranium thunbergii* SIEB. *et* ZUCC.

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SYNOPSIS

The effects of fertilizer levels on the growth and tannin accumulation in a medicinal plant, *Geranium thunbergii* were determined on the potted plant with both deficient and excessive fertilizer applications. The standard rate of three fertilizer nutrients was established at ratios of 1.6 g ammonium sulphate (N element), 1.4 g superphosphate (P element), and 0.7 g potassium sulphate (K element), per crop. A reduction of dry matter production was 30-50% in plants with deficient fertilizer rates, but tannin concentration in leaf blades did not differ. The application of nitrogen fertilizer stimulated the increase of dry weights, which were also increased with the compound fertilizer containing N, P, and K elements. At higher application rates of 5 to 10 times the amount of nitrogen from standard treatment increased the obvious yield of dry weight more than 2-fold to 4-fold compared with the standard. However, tannin concentration in leaf blades decreased about 5 to 23% in all excessive applications of fertilizer.

INTRODUCTION

Nutrient availability is one of the major factors which determine the productivity in agricultural and natural ecosystems. The wide variation of the amount of soil nutrients sometimes causes the nutrient deficiency for crops growing or brings about a toxic effect. The appropriate nutrient concentration has been described as the range of concentration that occurs between deficiency and luxury consumption.[1] Many data has been reported on the yield affected by nutrient levels in various crops. However, studies on the pharmacological yield of medicinal plants are limited. Thus, this study investigated broadly the influence of application rates of chemical fertilizer on the dry matter and tannin accumulation in a medicinal plant, *Geranium thunbergii*.

MATERIALS AND METHODS

Plant material and cultivation *Geranium thunbergii* plants were obtained from the Sayama medicinal plant garden of the Faculty of Pharmaceutical Sciences of Kinki University. In October 1991, the individual plants were devided by hand into the small plants, when the shoots started to grow near the base of the main stem. Leaves were removed to remain two or three young ones,

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and the roots were cut 2 to 3 cm long. These seedlings were planted in a plastic pot 6 cm diameter, filled with a mixture of vermiculite and sandy loam soil (1 : 1 ; v/v) and grown without fertilizer. At the start of the experiment, plants were selected for uniformity in size and 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).

The chemical fertilizer used in this study was ammonium sulphate [$NH_4(SO_4)_2$], superphosphate [$CaH_4(PO_4)_2$], and potassium sulphate [K_2SO_4] for the respective N-, P-, and K-sources. These fertilizers were grounded to used in a fine form. The standard rate of fertilizer applied to one pot per crop which was determined by the conventional recommendation of Fujita[2)], was 1.6 g of $NH_4(SO_4)_2$, 1.4 g of $CaH_4(PO_4)_2$, and 0.7 g of K_2SO_4. All plants were grown in a plastic greenhouse and watered thoroughly with tap water.

Experiment I : Deficient fertilizer application from January to July

This experiment was conducted from January to July, 1992. A half dose of standard treatment was applied to the plants with two ways ; F1 and F2, designated in Table 1. In these deficient fertilizer treatments, fertilizers were applied 3 times. The fertilizer was put into a small hole made between the plants. The amount of standard treatment (St) was divided into 10 parts for 6 applications which was applied every month. Five pots were used for each treatment.

Experiment II : Deficient fertilizer application from July to October

This experiment was conducted from July to October, 1992. The cultivating and maintenance methods were the same as the Experiment I. On July 9, the aerial portions were cut to leave the basal stem approximately 2-3 cm, which enabled the stem to grow new shoots from July to October. Similar amount of fertilizer as studied in Experiment I was applied 5 times in the standard treatment every two weeks and 3 times in F3, F4 treatments at the same rating of F1, F2 as shown in Table 1. Four pots were used for each treatment.

Experiment III : Excessive fertilizer application from May to November

This experiment was conducted from May to November, 1992. The excessive amounts of fertilizer were established at 2, 5, and 10 times of the standard treatment. Comparisons were made of the increasing rates of each element and compound fertilizer of N, P, and K elements. The fertilizer was applied every month using the same method of the standard treatment in Experiment I. The harvesting time was determined in November as the method described in the

Table 1. Scheme of fertilizer application in experiment I and II from january to october, 1992

Treatment[a)]	Month of fertilizer application									
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Experiment I										
Standard	1	2	2	2	2	1	*			
F1	1	2	2	0	0	0	*			
F2	1	2	0	0	0	2	*			
Experiment II										
Standard							2 2	2 2	2 0	*
F3							2 2	1 0	0 0	*
F4							2 2	0 0	0 1	*

[a)] The amount of standard treatment was divided into 10 parts for 6 applications, which was applied every month in the Experiment I. For Experiment II, similar dose of fertilizer was applied 5 times in the standard treatment every two weeks. In these deficient fertilizer treatments, a half dose of standard treatment was applied 3 times in the F1, F2, F3, and F4. See Materials and Methods for details.

* harvest time

measurement of plants. Four pots were used for each treatment.

Measurement of plant growth At harvest time in each experiment, all plants were cut at the soil surface level and separated into leaves, stems, and roots. The numbers of stems and leaves were counted. The length of stems and the third internode from the apex on those stems were measured. The dry weights of plant parts were determined after oven-drying at 40°C for 72 h. The dry weight ratio of top to root was calculated. In Experiment III, the number of mature pods was counted and collected as soon as they were completely brown or black (fully mature). The remaining pods on the plants at harvesting time were counted as immature pods.

Assay of tannin concentration Leaf blades from the third and fourth from the apex and whole stems were assayed for tannin in Experiment I and II. For experiment III, the total leaf blades on the one-year-old stems and on the new shoots emerged from the basal stems were separately assayed for tannin. Dried samples were grounded into a fine powder and 150 mg of leaves and 500 mg of stems were weighed for the analysis. The samples were extracted with 60% acetone and assayed for the amount of tannin in extracts as a percentage of the original dry weight using a colorimetric procedure of the relative affinity of tannin to methylene blue based on the method of Okuda and co-workers.[3] Tannic acid JP (Japanese Pharmacopoeia), Lot No. M3B 9578, was purchased from Nacalai Tesque, Inc., Kyoto, Japan, and used as the reference.

Statistical analysis Experiments were laid out in a completely radomized experimental design. All sets of data were subjected to ANOVA and significantly different means were separated by Duncan's multiple range test at $p=0.05$.

RESULTS

Experiment I The dry weight of plants which responded to standard treatment was significantly greater than F1 and F2 treatment (Fig. 1). The dry weight of the plants applied half the amount of fertilizer decreased conspicuously by less than 50% of the standard treatment. There was little difference in the number of stems and leaves between standard, F1, and F2 treatments (Table 2). The dry weight ratio had no statistical difference between treatments. The mean length of the three longest stems was significantly shorter in F1 treatment. The tannin concentration in leaf blades of plants under the influence of deficient fertilizer did not differ from those of the standard treatment (Fig. 2).

Experiment II As shown in Fig. 1, the growth responses of plants in October showed similar to those in Experiment I. The reduction of dry weight of aerial parts was 30-35% in the F3 and F4 treatments compared with the standard treatment. However, the root dry weight was not affected by the fertilizer application. The number of leaves and the dry weight ratio were significantly decreased in deficient fertilizer treatments (Table 2). The tannin concentration in leaf blades and stems did not differ between different fertilizer applications (Fig. 2).

Experiment III Figure 3 shows the effect of excessive fertilizer application on dry matter production. The dry weight of aerial parts increased with increase in the amount of nitrogen. Marked increases in the dry weight of these plants were obtained when the nitrogen element was applied at rates of 5 and 10 times. The dry weight was greatest in the plants applied 10 times (N : P : K) the standard treatment. It should be noted that the excessive applications of phosphorus and potassium did not increase the dry weight. The dry weight of roots was a little range of difference by excessive fertilizer. The increase of leaf and pod number were significantly greater than the 5-fold to 10-fold associated with consistently excessive nitrogen applied at 5 and 10 times (Table 3, and Fig. 4). The dry weight ratio increased about 2-fold by 5 times and 4-fold by 10 times application of nitrogen and compound fertilizer. On the contrary, tannin concentration decreased in the leaf blades both on one-year-old stems and new shoots (Fig. 5). The concentra-

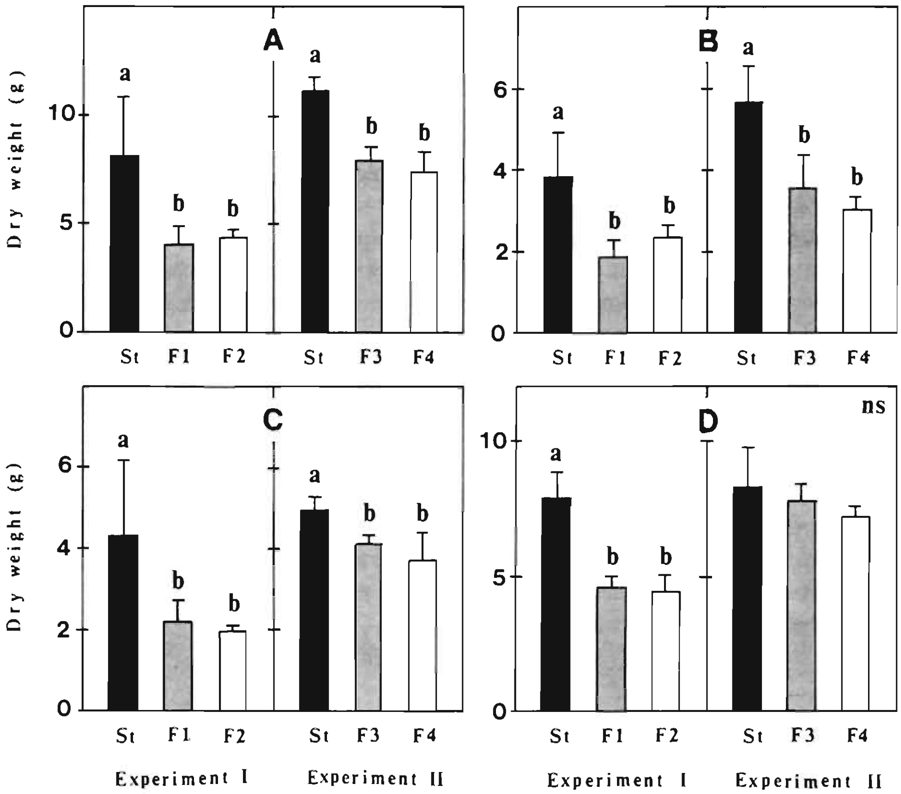

Fig. 1 Dry weights of aerial parts (A), leaves (B), stems (C), and roots (D) in the plants with fertilizer deficiency
Data are means ± SD of five, four replicates in Experiment I and II, respectively. Bars with different letters for values from the same experiment show significant differences by Duncan's multiple range test at p=0.05. ns, not significant.

tion in total leaves on the one-year-old stems decreased about 23% compared with the control (taken to be 100%) by 10 times application of nitrogen and compound fertilizer. The changes in tannin concentration in both assayed leaves were almost similar under different fertilizer applications, although the appearances of leaf colour and shape were greatly different.

DISCUSSION

The reduction of dry weight was clearly recognized under insufficient fertilizer application with a sustained lower growth rate. A difference in results between different applications of deficient fertilizer at the level of a half amount of standard treatment was not noticeable in these experiments. The responses of plants in dry weight yield related to fertilizer rates were conspicuously more than the ways of the application. Hale and Orcutt[4] suggested that yield or growth rate are usually impaired before other symptoms of deficiency occur so that indications of at least the potential for a deficiency must be considered in any diagnosis procedures. Our results showed that inhibited dry matter production by deficient fertilizer application did not reduce the tannin concentration as expressed in the standard treatment.

This experiment reveals that sufficient fertilizer rates brought about an increase of dry weight

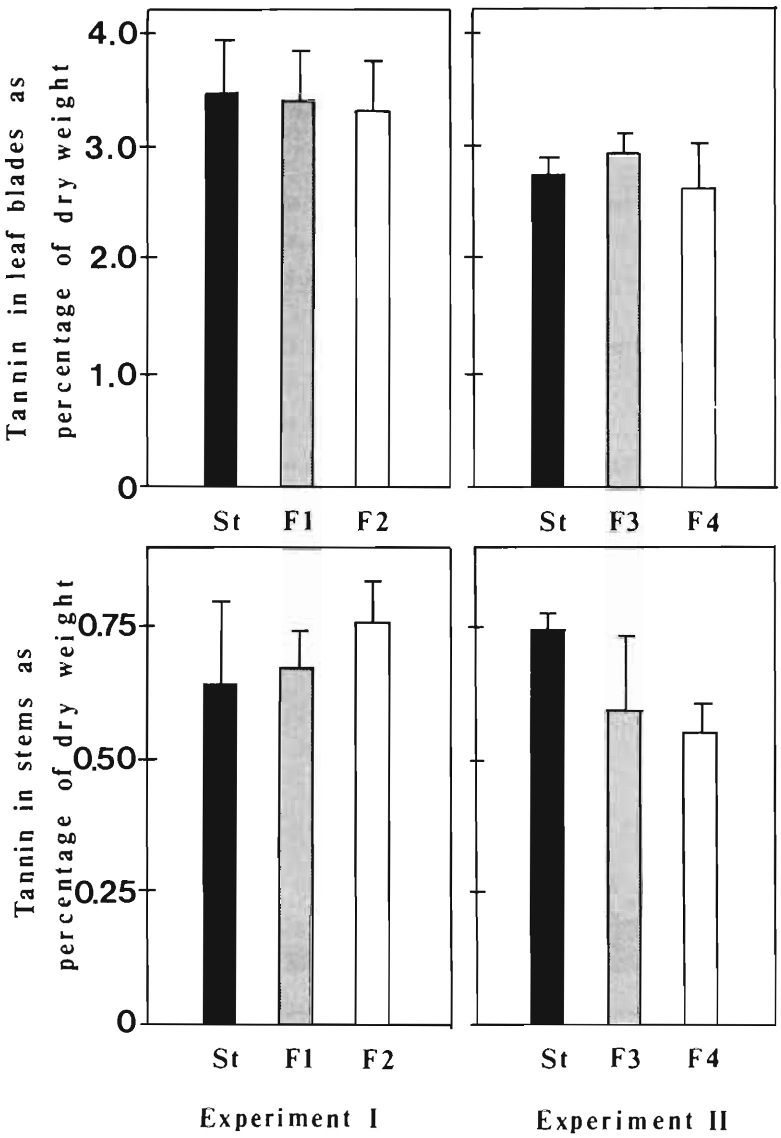

Fig. 2 Tannin concentration in leaf blades (upper), and stems (lower) in the plants with fertilizer deficiency
Bars represent means ± SD of five, four replicates in Experiment I and II, respectively.

with appreciable nitrogen effects. This encourages the effectives of supplementing with complete elements of N, P, and K. The increases in dry matter production were caused by nitrogen application, especially when the amount of nitrogen was increased 5 or 10 times. In this experiment, plant response without any toxicity or residual effects was achieved by increasing the amount of fertilizers gradually every month. Methods of supplying nitrogen are better matched to the growth of the plants and losses by leaching were also minimized. However, more efficient utilization of nitrogen fertilizer is possible through a balance of nutrients which would also contribute to a more efficient use of nitrogen as well as other nutrients.[4] Application of P or K

Table 2. Effects of deficient fertilizer applications on some morphological features

Treatment	Number of		Length of		Top / root ratio
	stems	leaves	stems[a] (cm)	internodes[b] (cm)	
Experiment I (n=5)					
Standard	5.2 ns	68.4 ns	27.00 a	3.73 ns	1.03 ns
F1	4.4	41.4	15.43 b	2.72	0.89
F2	3.8	45.6	20.07 ab	3.21	0.99
Experiment II (n=4)					
Standard	10.0 ns	195.5 a	22.29 ns	3.28 ns	1.40 a
F3	7.3	139.0 b	23.06	3.02	1.02 b
F4	7.0	138.5 b	20.59	3.69	1.02 b

Means with different letters for values from the same Experiment within columns show significant differences by Duncan's multiple range test at p=0.05. ns, not significant.
[a] mean of the three longest stems.
[b] on the third internode from the apex of the longest stem.

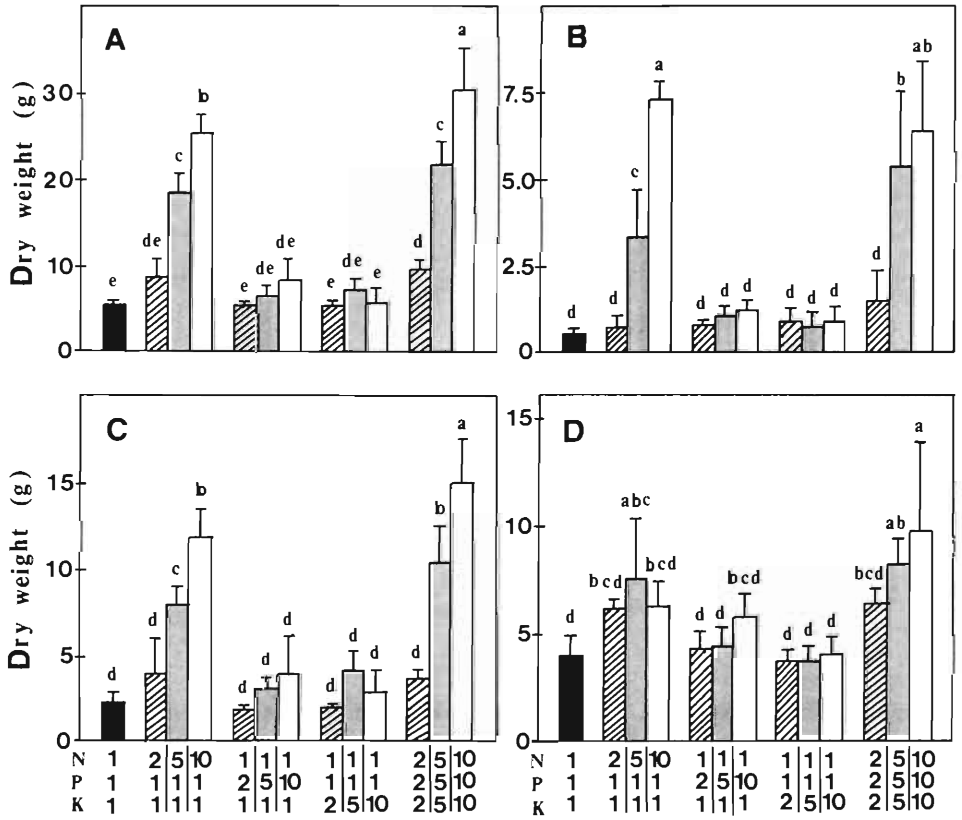

Fig. 3 Dry weights of aerial parts (A), leaves (B), stems (C), and roots (D) in the plants given excessive fertilizer
Data are means ± SD of four replicates. Bars with different letters show significant differences by Duncan's multiple range test at p=0.05.

Table 3. Effects of excessive fertilizer applications on some morphological features

Treatment	Number of		Length of	Top/root	Number of pods	
N:P:K	stems	leaves	stems[a] (cm)	ratio	mature	immature
1 : 1 : 1[b]	2.0 d	44.8 d	30.13 cd	1.42 cde	65.3 ef	7.4 b
2 : 1 : 1	2.8 d	54.5 d	32.58 abcd	1.39 de	103.0 cde	4.4 b
5 : 1 : 1	4.5 cd	227.0 c	36.88 abcd	2.78 bc	204.8 b	8.9 b
10 : 1 : 1	7.0 bc	564.0 a	44.42 a	4.26 a	188.9 b	8.4 b
1 : 2 : 1	3.0 d	37.3 d	30.38 cd	1.25 de	59.1 f	4.0 b
1 : 5 : 1	3.3 d	77.8 d	26.08 d	1.54 cde	63.1 ef	9.9 b
1 : 10 : 1	3.0 d	86.5 cd	35.23 abcd	1.47 cde	68.6 ef	10.1 b
1 : 1 : 2	3.3 d	44.5 d	28.21 cd	1.37 de	52.9 f	3.8 b
1 : 1 : 5	4.3 d	53.8 d	34.42 abcd	1.93 cde	54.0 f	8.5 b
1 : 1 : 10	3.3 d	65.8 d	34.06 abcd	1.39 de	40.5 f	8.4 b
2 : 2 : 2	3.0 d	93.5 cd	31.21 bcd	1.52 cde	120.9 cd	8.6 b
5 : 5 : 5	7.3 b	400.3 b	40.17 abc	2.76 bcd	130.1 c	40.9 a
10 : 10 : 10	10.5 a	471.8 ab	43.25 ab	3.69 ab	248.1 a	38.3 a

Values are means of four replicates. Means with different letters in the same column show significant differences by Duncan's multiple range test at $p=0.05$.
[a] mean of the three longest stems.
[b] standard treatment.

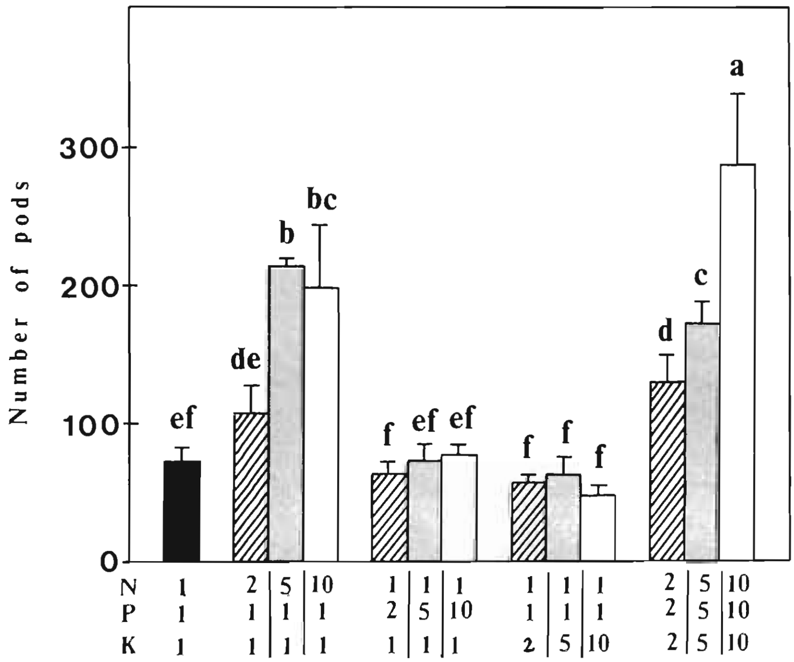

Fig. 4 Number of total pods in the plants given excessive fertilizer
Data are means ± SD of four replicates. Bars with different letters show significant differences by Duncan's multiple range test at $p=0.05$.

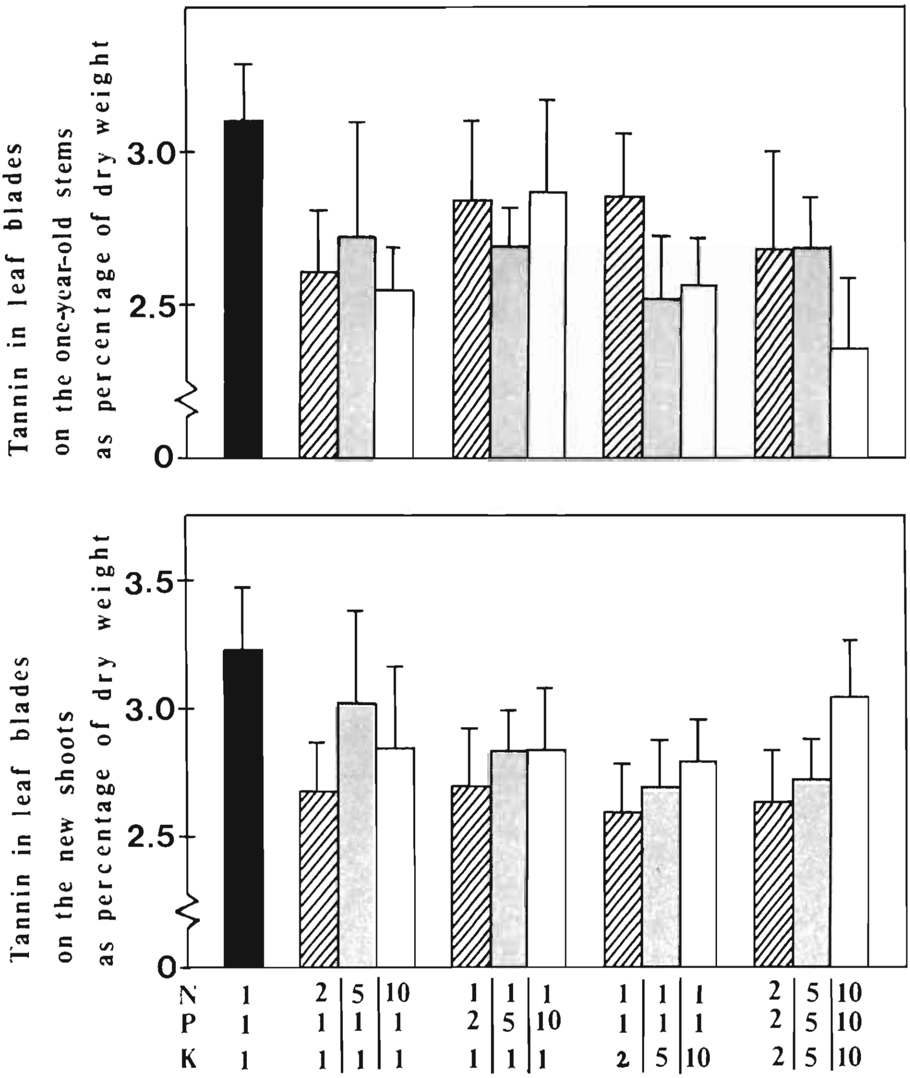

Fig. 5 Tannin concentration in leaf blades on the one-year-old stems (upper), and on the new shoots (lower) in the plants given excessive fertilizer
Bars represent means ± SD of four replicates.

elements alone over the treatments did not exhibit beneficial enhancement of dry matter production.

Tannin concentration in leaf blades reduced by excessive nitrogen application. Neera *et al.*[6] studied *in vitro* cultures of *Sapium sebiferum* and showed that a large amount of geraniin was produced in the callus cultured of synthetic medium without NH_4NO_3. The addition of nitrogen sources to the medium was not effective to produce tannin. This suggests that fertilizer manipulation to increase plant dry weight may be feasible by the applying high rates of nitrogen, which also results in reducing the concentration of tannin in leaf blades when considered in dry weight.

In conclusion, appling chemical fertilizer application stimulated the dry matter production of

Geranium thunbergii. The application of deficient fertilizer clearly decreased in dry weight. On the other hand, increasing the fertilizer rate, especially the amount of nitrogen, had important influences for obtainable dry weight. This plant exhibited a sensitive response to nitrogen application, which produced a larger yield by adding 5 or 10 times the compound fertilizer of standard treatment. However, tannin concentration in leaves decreased by adding chemical fertilizer.

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N-P-K 施肥量が薬用植物，ゲンノショウコ，の成育と収量に及ぼす影響

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要　約

薬用植物の栽培においては，利用植物体の増収に加えて，その薬効成分含量の増加とさらに特に臨床の面から，それらの含有率の安定化が望まれている。

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ついて，栽培条件のうち施肥条件を大きく変えた場合に本薬草の成育と収量に及ぼす影響を調べた。

慣行施肥量を対照区としてその半量の施肥区では，本植物の成長量（地上部乾重）は約半分に減少したが，葉のタンニン含量はほとんど変わらなかった。また施肥量，特にN要素を5倍，10倍にして与えると，成長量は2～4倍に増加したが，タンニン含量はやや減少して，対照植物のおよそ77～95％となった。これらの結果よりゲンノショウコの成育は，施肥量により大きく変化するがタンニン含量の変化は比較的に少ないと考えられる。