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Evaluation of Allelopathy in *Crotalaria* by Using a Seed Pack Growth Pouch*

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Abstract : Allelopathic effect of aqueous extracts of six *Crotalaria* species on wheat root growth at early growing stage was examined by using a seed pack growth pouch. There was a significant difference in total root length of wheat with application of the extract of each species compared with control at 21 days after planting. *C. juncea* and *C. pallida* suppressed the length by approximately 40% based on the control. Remarkable suppression of root growth could be clarified by image-processing of the root system which appeared on the surface of the pouch. A significant reduction in the length of the longest root was also observed, and *C. juncea* and *C. spectabilis* showed severe reduction. Definite inhibition was observed with the leaf extract compared with the stem extract in *C. spectabilis*, and the inhibition was remarkable as the concentration of the extracts increased. Dry weights of both top and root of wheat were not influenced by application of *C. brevidens*, *C. juncea*, *C. lanceolata* and *C. pallida*. With application of *C. spectabilis*, however, top dry weight was restricted to a low value compared with the control. Fractal dimension of the profile of root system ranged in value from 1.27 to 1.35, and it was not necessarily influenced by the application of extract of each species. These results indicated that wheat root growth was inhibited by application of the aqueous extract of *Crotalatia*, and the seed pack growth pouch techique might be applied to the evaluation of allelopathy.
Key words : Allelopathy, *Crotalaria*, Fractal analysis, Root system, Seed pack growth pouch, *Triticum aestivum* L., Wheat.

グロースポーチ法によるクロタラリア属植物のアレロパシーの評価 : 大段秀記・大門弘幸・三本弘乗（大阪府立大学農学部）

要　旨 : マメ科緑肥作物として導入が試みられているクロタラリア属植物（*Crotalaria* spp.）6種の葉および茎の搾汁液が後作物としてのコムギの根系の初期生育に及ぼす影響をグロースポーチを用いた方法で調査した．生葉10 g を培養液中で摩砕して調整した搾汁液を施用したところ，施用後21日目において，総根長では，各処理区が培養液のみを施用した対照区に比べて有意に短くなり，*C. juncea* 区と *C. pallida* 区では対照区の40%にまで抑制された．最長根長においても有意な抑制が認められ，*C. juncea* 区と *C. spectabilis* 区で特に顕著であった．*C. spectabilis* の施用においては，葉の搾汁液が茎の搾汁液よりも強い抑制を示し，10 g 区に比べて20 g 区が抑制程度が大きかった．地上部および地下部乾物重においては，*C. brevidens* 区，*C. juncea* 区，*C. lanceolata* 区，*C. pallida* 区では明確な影響が認められなかったが，*C. spectabilis* 区では地上部乾物重が対照区に比べて低い値を示した．コムギの根系構造のフラクタル次元は1.27から1.35の値を示し，搾汁液の施用の影響は認められなかった．以上のように，クロタラリア属植物の搾汁液はコムギの根の生長を抑制し，その程度に種間で差異が認められた．本研究で用いたグロースポーチ法は，根系を容易に観察できることから生育初期におけるアレロパシーの新しい評価法として期待できると思われる．
キーワード : アレロパシー，クロタラリア，グロースポーチ，コムギ，根系，フラクタル解析．

Crotalaria spp. have been recently expected to be introduced as green manure legumes, because of its high dry matter production potential and vigorous growth on poor soil[1,13,14,18]. We have already reported that *C. juncea* contributed to the succeeding wheat in regard to the supply of nitrogen[18]. In applying green manure legumes, the recovery of fixed-nitrogen by the succeeding crops is generally recognized. However, an allelopathic inhibitory effect on the growth of the succeeding crop due to chemical compounds released from incorporated plant materials should be also considered.

Plant residues incorporated into soil and aqueous extracts of plant forages have been reported to act inhibitory on seedling growths in some plant species such as alfalfa (*Medicago sativa* L.)[7] and velvet bean (*Mucuna pruriens* L.)[4]. In order to survey the allelopathic inhibitory activity of many plant species, measuring the length of radicle, which elongated into the

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agar solidified medium poured into a plant box or a cell well[5,6,9] containing plant materials or the extracts, was also attempted by using lettuce (*Lactuca sativa* L.) as a test plant. In these cases, remarkable inhibition on the growth of lettuce radicle was observed in application of fresh and defoliated leaves. However, there have been little informations on the allelopathic inhibition of development of root system.

For investigating a root system of rice plant at early growing stage, applied was a seed pack growth pouch wich is often used for nodulation test in legumes[8]. The profile of root system grown in the pouch could be easily observed.

In this study, considering the *Crotalaria*-wheat succesive corpping system, the effect of aqueous extract of *Crotalaria* plant on root growth in wheat at early growing stage was investigated, and also emphasised was the application of a seed pack growth pouch method for observing a profile of root system influenced by allelopathic inhibition.

Materials and Methods

1. Plant materials

C. brevidens, *C. juncea*, *C. lanceolata*, *C. pallida*, *C. sessiliflora* and *C. spectabilis* were used as materials for aqueous extracts.

These six species were grown on the Experimental Farm at University of Osaka Prefecture in Sakai. Soil at the site was a silty loam (a gray lowland soil ; Haplaquept) with a pH (H_2O) of 5.6 and a EC of 23 μScm^{-1}. The seeds were sown on May 11 in 1993. Each plot was 1.3×2.9 m in area with 4 rows and planting distance was 0.3 m. Ammonium sulfate, superphosphate and potassium sulfate were applied in each plot at the rate of 3 g N, 10 g P_2O_5 and 10 g K_2O per m^2.

2. Preparation of aqueous extract

Fresh leaves of each species were sampled during October 1993, when they had numerous flowers and some pods. In all of six species, ten grams of the fresh leaves were homogenized by a homogenizer (Nihonseiki Kaisya LTD., Japan) at 12,000 rpm for 10 min with 30 m*l* of Tadano's and Tanaka nutrient solution[11]. The homogenates were filtered through a gauze and the filtrates were filled up to 300 m*l* with the same nutrient solution. The pH of the homogenate was adjusted to 5.8—6.0 after filling up to a constant volume. This solution was used as the extract.

To compare with leaves and stems on the inhibitory activity, the aqueous extracts were also prepared from 10 or 20 g each of leaves and stems of *C. spectabilis* by the same method described above.

3. Bioassay of the aqueous extract by a seed pack growth pouch

Thirty m*l* of the aqueous extract was put into a seed pack growth pouch (17.8×16.5 cm, Mega International, USA). Wheat (*Triticum aestivum* L. cv. Norin 61) seeds were surface-sterilized in sodium hypochlorite solution (1% active chlorine) for 15 min and rinsed 3 times with sterilized water. These seeds were placed on a filter paper (No. 2, ADVANTEC, Toyo, Japan) wetted by distilled water in a petri dish. The dishes were incubated in the light of 20 μmol • photons s^{-1} m^{-2} at 25°C. After 3 days, the seedling was transferred to the pouch. The pouches inoculated the seedlings were arranged in a rack for shading roots and incubated in a light/dark cycle of 9/15 h (50 μmol • photons s^{-1}m^{-2}) at 25°C. 10 m*l* of distilled water was put into the pouches every two days for keeping the solution volume constant during the experiment. As control, only nutrient solution was applied.

At 21 days after transferrence, plant height, the number of leaves, and the length of the total root and the longest root were measured. Total root length was estimated by counting the pixels constituting the processed root image as described after. The sampled plants were oven-dried at 70°C for 3 days and then dry weight was measured. The experiment was conducted with 10 pouches of each treatment.

4. Evaluation of root system using an image processing system

The profile of root system which appeared on the pouch surface was made a photocopy and then the image of root system was digitized with an image scanner (Color OneScanner, Apple Computer Inc., USA). It was processed with a personal computer (Color Classic II, Apple Computer Inc., USA). The processed root image was used for estimating total root length and analysing the fractal dimension. The fractal dimension, D value, ranged from 0.35 to 5.6 mm in length of a side

of pixel as scaling factor was measured according to the method as described by Tatsumi et al.[16].

Results and Discussion

Table 1 shows the growth of wheat plant grown in the seed pack growth pouch applied the aqueous extracts from fresh leaves of *Crotalaria* species at 21 days after transferrence. There were no significant differences on plant height and the number of leaves of wheat among the application of the extracts of different *Crotalaria* species. Dry weights of both top and root of wheat were not influenced by application of *C. brevidens*, *C. juncea*, *C. lanceolata* and *C. pallida*. With application of *C. spectabilis*, however, top dry weight was restricted to low value compared with the control.

There was a significant difference in total root length of wheat with application of the extract of each species compared with control. Based on the control, *C. juncea* and *C. pallida* suppressed the length by approximately 40%. A significant reduction in the length of the longest root was also observed, and *C. juncea* and *C. spectabilis* showed severe reduction as 11.3 and 13.4 cm respectively.Considering no differences in pH and EC between each extract solution and the control (data not shown), it is suggested that the inhibition on wheat root growth was due to allelopathic substances in the extract of each *Crotalaria* species.

Some plant species have been reported to have a number of phytotoxic compounds such as ferulic acid[10,17] and chlorogenic acid[15,17]. Authentic chlorogenic acid showed the inhibition of growths of test plants as well as the extracts of alfalfa (*Medicago sativa* L.)[7], perennial buckwheat (*Fagopyrum symosum* Meissn.)[17] and taro (*Colocasia esculenta* L.)[12]. For

Table 1. Effect of the aqueous extracts of leaves in *Crotalaria* spp. on the growth of wheat grown under the culture condition by a seed pack growth pouch at 21 days after transferrence.

Species	Plant height (cm)	No. of leaves	Dry weight (mg)		Total root length (cm)	Length of the longest root (cm)
			Top	Root		
C. brevidens	22.7	2.9	16.8	7.1	200	15.9
C. juncea	23.9	2.7	16.8	7.3	190	11.3
C. lanceolata	19.5	2.8	14.3	7.6	228	15.2
C. pallida	20.4	2.7	14.5	7.3	193	16.0
C. sessiliflora	23.0	2.7	15.3	8.4	260	15.1
C. spectabilis	18.7	2.6	13.1	7.8	215	13.4
Control	21.2	2.7	17.0	7.1	320	24.4
LSD (P=0.05)	3.7	0.2	2.9	1.2	61	3.3

Data : mean of ten replicates.

Table 2. Effect of the aqueous extracts of leaves and stems in *C. spectabilis* on the growth of wheat grown under the culture condition by a seed pack growth pouch at 21 days after transferrence.

Plant part	Conc. (g/300ml)	Plant height (cm)	No. of leaves	Dry weight (mg)		Length of the longest root (cm)
				Top	Root	
Leaf	10	19.8	2.6	13.9	8.1	13.9
	20	16.9	2.6	13.0	7.6	12.4
Stem	10	21.2	2.5	15.3	7.9	21.2
	20	19.2	2.5	14.3	8.3	18.1
Control		22.1	2.8	19.4	7.7	27.8
LSD (P=0.05)		3.6	0.3	2.9	1.2	3.4

Data : mean of ten replicates.

detecting the interspecific differences on the inhibitory aspects in the present experiment, studies on identification of the allelopathic chemicals in *Crotalaria* species is now in progress.

Table 2 shows the growth of wheat plant grown in the pouch applied the aqueous extracts prepared from leaves and stems of *C. spectabilis* at 21 days after transferrence. Application of the extract remarkably depressed the top dry weight and the length of the longest root of wheat as same as described in Table 1.

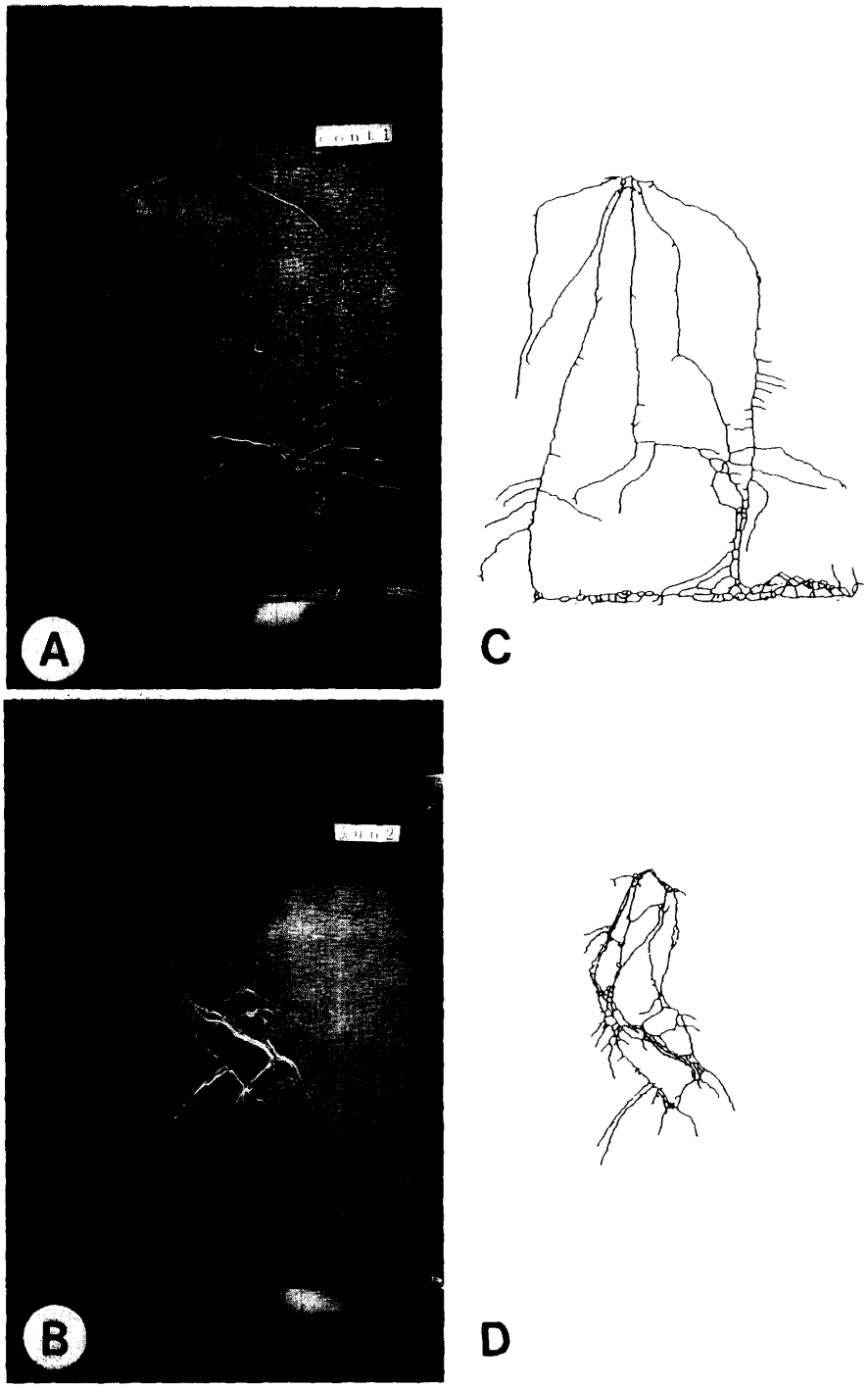

Fig. 1. The profile of root system of wheat grown in a seed pack growth pouch for 21 days in control (A) and in application of the aqueous extract of *C. juncea* leaves (B), and their digitized images (C, D).

Based on the control, the length was suppressed by 24% with 10 g of stem to 55% with 20 g of leaf.

In *Polygonum orientale*[2] and *Clerodendrum viscosum*[3], the leaf extract has been previously reported to show more severe suppression to the root growths of some test plants compared with the other organs such as stem and flower. In the present experiment, the definite inhibition was also observed with the leaf extract compared with the stem extract in *C. spectabilis*, and the inhibition was remarkable as the concentration of the extract increased. It is suggested that the allelopathic substances might be largely involved in leaf and severe inhibition with application of leaf would be caused by an increase in the amounts of them.

Residual parts of taro have been reported to inhibit the seminal root development of sorghum (*Sorghum bicolor* L.) grown by using a root box culture technique[12]. Taro residue could modify the root system morphology of sorghum with a drastic suppression on the length of lateral root in the zone containing the residue. Since root function is related to the root system morphology, analysis of the inhibitory effect on the root system would be important in the study of allelopathy.

Fig. 1 shows the root system of wheat plant appeared on the surface of the pouch at 21 days after transferrence. Remarkable suppression of root growth in application of the extract could be observed by digitizing the photocopied-image of the root on the image processor. It has been demonstrated that the profile of root system was fractal in several crops and the fractal dimension, D value, was considered as one of the indicators of intricacy of root system, i.e. highly branching structure[16]. In this study, D value was not necessarily influenced by the application of the extract of each species, and it ranged in value from 1. 27 to 1.35 as described in Table 3. Therefore, the root system of wheat applied the extract of *Crotalaria* species would not be intricate after 3 weeks of culture. However, significant differences in the total root length and the length of the longest root were definitely found. These quantitative alteration in root system would influence the fractal dimension and the function as a root system on the late growing stages.

In conclusion, remarkable supperssion on wheat root growth in application of extracts of *Crotalaria* spp. could be found. A seed pack growth pouch technique would be applicable for evaluating an allelopathic inhibition on early growing stage in some plant species, since it is rather convenient for culture and useful for detecting the profile of root system.

Table 3. Effect of the aqueous extracts of leaves in *Crotalaria* spp. on the fractal dimension estimated by digitizing the root system of wheat.

Species	Fractal dimension
C. brevidens	1.27
C. juncea	1.32
C. lanceolata	1.33
C. pallida	1.35
C. sessiliflora	1.29
C. spectabilis	1.35
Control	1.32
LSD(P=0.05)	0.04

Data : mean of ten replicates.

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