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

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Received January 31, 1995

**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. brevifrons*, *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 *Crotalaria*, and the seed pack growth pouch technique might be applied to the evaluation of allelopathy.

**Key words**: Allelopathy, *Crotalaria*, Fractal analysis, Root system, Seed pack growth pouch, *Triticum aestivum* L., Wheat.

*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 on inhibitory seedling growths in some plant species such as alfalfa (*Medicago sativa* L.)[^71] 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...
agar solidified medium poured into a plant box or a cell well 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 which is often used for nodulation test in legumes. The profile of root system grown in the pouch could be easily observed.

In this study, considering the Crotalaria-wheat successive cropping 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. brevifrons, 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; Haplaquert) with a pH (H2O) of 5.6 and a EC of 23 $\mu$S cm$^{-1}$. The seeds were sown on May 11 in 1993. Each plot was 1.3 $\times$ 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 P2O5 and 10 g K2O 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 ml of Tadano’s and Tanaka nutrient solution$^{11}$. The homogenates were filtered through a gauze and the filtrates were filled up to 300 ml 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 ml of the aqueous extract was put into a seed pack growth pouch (17.8 $\times$ 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 $\mu$mol • 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 $\mu$mol • photons s$^{-1}$m$^{-2}$) at 25°C. 10 ml 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 transference. 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 acid10,17 and chlorogenic acid15,17. Authentic chlorogenic acid showed the inhibition of growths of test plants as well as the extracts of alfalfa (Medicago sativa L.)71, perennial buckwheat (Fagopyrum symosum Meisn.)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 transference.

<table>
<thead>
<tr>
<th>Species</th>
<th>Plant height (cm)</th>
<th>No. of leaves</th>
<th>Dry weight (mg)</th>
<th>Total root length (cm)</th>
<th>Length of the longest root (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Top</td>
<td>Root</td>
<td></td>
</tr>
<tr>
<td>C. brevidens</td>
<td>22.7</td>
<td>2.9</td>
<td>16.8</td>
<td>7.1</td>
<td>200</td>
</tr>
<tr>
<td>C. juncea</td>
<td>23.9</td>
<td>2.7</td>
<td>16.8</td>
<td>7.3</td>
<td>190</td>
</tr>
<tr>
<td>C. lanceolata</td>
<td>19.5</td>
<td>2.8</td>
<td>14.3</td>
<td>7.6</td>
<td>228</td>
</tr>
<tr>
<td>C. pallida</td>
<td>20.4</td>
<td>2.7</td>
<td>14.5</td>
<td>7.3</td>
<td>193</td>
</tr>
<tr>
<td>C. sessiliflora</td>
<td>23.0</td>
<td>2.7</td>
<td>15.3</td>
<td>8.4</td>
<td>260</td>
</tr>
<tr>
<td>C. spectabilis</td>
<td>18.7</td>
<td>2.6</td>
<td>13.1</td>
<td>7.8</td>
<td>215</td>
</tr>
<tr>
<td>Control</td>
<td>21.2</td>
<td>2.7</td>
<td>17.0</td>
<td>7.1</td>
<td>320</td>
</tr>
</tbody>
</table>

LSD (P = 0.05) 3.7

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 transference.

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Conc. (g/300ml)</th>
<th>Plant height (cm)</th>
<th>No. of leaves</th>
<th>Dry weight (mg)</th>
<th>Length of the longest root (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Top</td>
<td>Root</td>
</tr>
<tr>
<td>Leaf</td>
<td>10</td>
<td>19.8</td>
<td>2.6</td>
<td>13.9</td>
<td>13.9</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>16.9</td>
<td>2.6</td>
<td>13.0</td>
<td>12.4</td>
</tr>
<tr>
<td>Stem</td>
<td>10</td>
<td>21.2</td>
<td>2.5</td>
<td>15.3</td>
<td>21.2</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>19.2</td>
<td>2.5</td>
<td>14.3</td>
<td>18.1</td>
</tr>
<tr>
<td>Control</td>
<td>22.1</td>
<td>2.8</td>
<td>19.4</td>
<td>7.7</td>
<td>27.8</td>
</tr>
</tbody>
</table>

LSD (P = 0.05) 3.6

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 transference. 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.

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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* and *Clerodendrum viscosum*, 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. Tarso 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 transference. 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, was considered as one of the indicators of intricacy of root system, i.e. highly branching structure. 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 suppression 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.

**Acknowledgements**

We thank Dr. J. Tatsumi, Faculty of Agriculture, Kobe University, for advice on fractal analysis, and Dr. Y. Kujira, Faculty of Education, Kanazawa University, for information on a seed pack growth pouch. We are also grateful to the Chiba Prefectural Agricultural Experiment Station for supplying the seeds of *Crotalaria*.

**References**

5. ——— 1992. Establishment of allelopathy test

* In Japanese.
** In Japanese with English summary.
*** Translated from Japanese by the present authors.