# Allelopathy of Velvetbean: Its Discrimination and Indentification of L-DOPA as a Candidate of Allelopathic Substances

## Yoshiharu FUJII, Tomoko SHIBUYA and Tamaki YASUDA

Department of Environmental Biology, National Institute of Agro-Environmental Sciences (Tsukuba, Ibaraki, 305 Japan)

#### Abstract

A series of the studies conducted through the laboratory bioassays and field tests to search for allelopathic plants indicated that among the 70 plant species tested, velvetbean (Mucuna pririens var. utilis) was the most promising candidate. It is recognized that velvetbean, which is a tropical legume grown for green manure, has a special ability to smother weeds. The studies placed emphasis on evaluation of allelopathic properties of velvetbean. The field test showed the smallest weed population in the velvetbean stand plots as compared with the stand plots of tomato, eggplant, upland rice and fallow conditions. By means of HPLC and bioassay on germination and seeding growth tests, the substance inhibiting plant growth was identified as L -3,4-dihydroxyphenylalanine (L-DOPA). L-DOPA is a well-known substance biologically active in animal physiology, such as a precursor of neurotransmitter dopamine, and an intermediate of many alkaroids. The present studies revealed that L-DOPA contained in a large amount (about 1% of the fresh weight in leaves and roots of velevetbean) inhibited the growth of some companion plants, and that it probably contributed to its allelopathy. L-DOPA suppressed the growth of some broad leaf weeds, while little effect was observed on gramineous plants. In addition to its effectiveness as a green manure, velvetbean could be utilized as an allelopathic crop to control weeds.

Discipline: Soils, fertilizers and plant nutrition/Weed control Additional key words: companion plants, green manure, intercropping, phytotoxicity, weed control

## Introduction

Velvetbean (*Mucuna pruriens* (L.) DC. var. *utilis* or *Stizolobium deeringianum* Piper et Tracy, a synonym but now not recommended for use) is a tropical legume, grown generally for green manure and mixed culture. It is recognized that velvetbean increases the yield of its companion gramineous crops and that it smothers the growth of harmful weeds such as nutsedge (*Cyperus* spp.) and alang-alang (*Imperata cylindrica*)<sup>20,28</sup>). A series of experiments have been undertaken with bioassay for the purpose of screening allelopathic plants with special emphasis placed on chemical interactions among them. The results of those experiments indicated that velvetbean

was the most promising candidate  $\operatorname{crop}^{10,16}$ . A field test showed that the velvetbean stands minimized a size of weed population as compared with those of tomato, eggplant, upland rice and fallow<sup>8,15)</sup>.

The genus *Mucuna* consists of 100–200 species growing in the tropics and subtropics<sup>29,33)</sup>. There are two subgenera in *Mucuna*: one is *Mucuna* which is perennial and woody, and the other is *Stizolobium* which is annual or biennial and herbaceous. Cultivars belong to *Stizolobium*, the total plants of which are utilized for green manure and/or cover crop, their leaves for fodder, their grains for food and seeds, and their stems for medicine in Africa and China<sup>32)</sup>. Grain yield reaches as high as 1.5–2.0 t/ha, and fresh leaves and stems weigh 20–30 t/ha, indicating that velvetbean is one of the most productive crops in the world. If the physiological mechanism of its allelopathic activities are identified, the use of velvetbean could be further developed: cultivation in larger areas in the tropics, and greater utilization as green manure and/or weed-control crops. The present paper attempts to review the results of the studies on allelopathic activities of *Mucuna pruriens* and its association with allelopathic chemicals.

#### Materials and methods

(1) Survey on allelopathic plants<sup>9,10,16)</sup>

Approximately 70 plant species were tested on their allelopathy with a Richards' function method<sup>26)</sup>, which proved to be suited to germination tests of lettuce and some weed plants<sup>19)</sup>. In order to destroy the enzymes which degradate some constituents of a plant, and to minimize changes of the organic chemicals contained, the leaves, stems and roots were dried at 60°C for 24 hr. One hundred mg of the dried samples each was extracted with 10 ml of water. Extraction mixtures were sonicated for 60 sec to complete the migration of chemicals. The extracts was filtered with Whatman No. 4 filter papers. Ten lettuce seeds were placed in 4.5 cm-diameter petri dishes containing 0.5 ml of test solution on Whatman No. 1 filter papers. Those seeds were incubated at 25°C under a dark condition. The number of the germinated seeds was counted and hypocotyl and radicle growth were measured on the 4th day. The parameters for germination tests were: final germination percentage (A), germination rate (R), and the onset of germination (Ts)<sup>19)</sup>. A simplex method was applied to the computer simulation of germination curves with the Richards' function.

(2) Velvetbean cultivar

A dwarf cultivar of velvetbean, *Mucuna pruriens* var. *utilis* cv. anã, was used for the field test and the extraction of allelochemicals. The origin of this seed is Brazil.

(3) Incorporation of velvetbean leaves into soil

Two types of velvetbean were added separately to the volcanic ash soil in Tsukuba: one was dry leaves oven-dried at 60°C over night, and the other was fresh leaves. Dried plant residues, equivalent to 1 g in fresh weight, were added to 100 g of soils. The same weight of cellulose powder was added to other pots as a control. Fertilizers added to each pot were as follows: N, P, K, of 50, 100, 50 mg/100 g soil, respectively. Nitrogen content contained in the velvetbean residues (1.2%) was applied in compensation for loss.

(4) Weed appearance in the fields of velvetbean stands<sup>8)</sup>

Planting of velvetbean and some other plants was repeated for a period of 2 to 3 years. Plants were grown in a lysimeter, where the surface soils of 10 cm depth were replaced with uncultivated soils in the starting year. Each plot received a standard level of fertilizers except for fallow.

(5) Mixed culture of velvetbean with allelopathy discrimination methods

Allelopathy of velvetbean in the field was confirmed using stairstep<sup>12,13,34</sup>) and substitutive experiments<sup>14,15,17</sup>). The stairstep experiment was designed according to the method of Bell and Koeppe<sup>2</sup>). The substitutive experiment was modified from the methods of the relevant references<sup>14,15,17</sup>).

(6) Isolation and identification of alellopathic substances<sup>11</sup>

Some fractions were extracted from fully expanded leaves and roots of velvetbean with 80% ethanol. The acid fraction of the extract inhibited the growth of lettuce seedlings. This fraction was subjected to silica gel column chromatography and HPLC with an ODS column, and the major inhibitor was identical to L-3,4-dihydroxyphenylalanine (L-DOPA). The identity was established by co-chromatography with an authentic sample using two HPLC column systems (silica gel and ODS) equipped with an electro-conductivity detector.

#### **Results and discussion**

(1) Survey of allelopathic plants

Some 70 plants were investigated with lettuce seed germination tests in order to search for allelopathic plants. It was observed that the activity of velvetbean was distinctive (Table 1).

(2) Incorporation of velvetbean leaves into soil

An experiment was carried out to examine the effects of velvetbean on the growth of other plants in a mixed culture. The treatment also included an incorporation of velvetbean leaves into soils. Fresh leaves incorporation to soils (1.0% W/W in dry weight equivalent) reduced the emergence of succeeding kidney bean (*Phaseolus vulgaris*) to 60%, the plant growth to 30% of the control (Table 2).

-	Germination test					Growth test		Extraction	
Plant (part) <sup>a)</sup>	A <sup>b)</sup>	R <sup>c)</sup>	Ts <sup>d)</sup>	I c)	T50 <sup>f)</sup>	Hypocotyl <sup>g)</sup>	Radicle <sup>g)</sup>	ratio <sup>h)</sup>	
Compositae									
Ambrosia elatior (S)	94	74	2.1	34	1.6	139	54	10	
Ambrosia elatior (R)	87	141	1.7	78	1.2	146	63	10	
Taraxacum officinale (S)	97	37	0.4	94	1.3	105	79	6.25	
Taraxacum officinale (R)	99	32	0.5	64	1.6	108	66	10	
Solidago altissima (L)*	67	39	1.4	19	1.5	90	70	25	
Solidago altissima (R)	89	59	1.3	42	1.3	109	78	25	
Erigeron canadensis (L)	89	80	1.3	56	1.2	114	50	25	
Erigeron canadensis (R)	94	66	1.2	55	1.2	121	67	25	
Saussurea carthamoides (S)	97	74	2.1	34	1.7	139	63	10	
Saussurea carthamoides (R)	90	78	2.1	36	1.7	112	64	10	
Helianthus tuberosus (S)	91	96	1.4	62	1.3	104	67	25	
Helianthus tuberosus (R)	94	99	1.3	71	1.2	114	63	25	
Artemisia princeps (S)***	65	20	2.9	5	3.3	51	50	20	
Senecio vulgaris (W)	86	70	1.4	62	1.8	104	67	10	
Ixeris debilis (W)	85	96	1.3	71	1.6	114	63	10	
Carthamus tinctorius (W)	100	173	0.9	206	0.7	141	65	8	
Helianthus annuus (S)*	86	38	1.2	27	1.5	102	33	10	
Helianthus annuus (R)	100	191	1.2	167	0.7	130	52	12.5	
Gramineae									
Alopecurus geniculatus (S)	95	89	2.5	34	1.8	138	62	10	
Alopecurus geniculatus (R)	91	78	1.8	39	1.5	127	94	10	
Digitaria sanguinalis (S)	90	25	1.6	15	2.1	98	42	10	
Digitaria sanguinalis (B)	91	41	1.5	23	1.6	97	96	25	
Sasa sinensis (S)	94	55	3.2	17	2.7	134	44	25	
Miscanthus sinensis (S)	97	70	3.3	20	3.4	118	52	25	
Sorehum sudanense (S)*	86	66	1.3	47	1.3	107	31	10	
Sorphum sudanense (B)	100	132	1.0	135	0.8	106	58	12.5	
Sorghum bicola (S)	85	60	1.3	39	1.3	104	55	10	
Sorshum bicola (R)*	98	131	1.0	133	0.8	84	43	12.5	
Oryza satiya (1.)	100	226	2.2	105	1.0	114	77	12.5	
Hordeum vulgare (L)	100	102	0.9	114	1.0	144	65	6.3	
Hordeum vulgare (R)**	99	84	1.4	62	1.3	72	36	25	
Secale cereale (L)**	91	62	1.2	48	1.3	79	21	10	
Secale cereale (R)	100	186	1.4	142	0.8	132	55	12.5	
Avena sativa (L)	98	117	1.4	88	1.0	105	105	2.5	
Avena sativa (R)	98	84	1.2	70	1.2	131	126	5	
Leguminosae	2.262.0	20	2422.1	1.1720	0260	10.00	1000	16	
Pueraria lobata (L)**	82	72	5.0	12	2.2	73	45	12.5	
Pueraria lobata (Stem)*	98	57	3.4	17	3.5	111	32	10	
Pueraria lobata (R)	95	32	0.5	103	1.4	95	68	10	
Mucuna pruriens (L)***	96	82	9.3	9	4.6	79	26	25	
Mucuna pruriens (Stem)	96	45	1.1	38	1.6	96	54	10	
Mucuna pruriens (R)	95	98	1.8	49	1.1	95	51	6	
Arachis hypogaea (L)*	83	90	4.9	16	1.8	98	60	10	
Arachis hypogaea (R)	94	93	3.3	21	1.9	97	57	16	
Glycine max (S)	96	44	0.6	70	1.4	117	41	10	
Trifolium repens (S)	98	49	1.8	28	1.9	105	56	10	
Lupinus albus (S)*	95	98	2.8	33	1.6	100	37	12.5	
Pisum sativum (S)	99	45	0.5	99	1.1	115	38	10	
Vicia angustifolia (S)*	97	60	3.6	16	2.8	126	22	6.67	
Vicia hirsuta (S)*	100	62	3.6	18	2.8	114	24	6.67	

Table 1. Screening of allelopathic plants with lettuce germination/growth test

(continue)

# (Table 1, continued)

	Germination test					Growth test		Extraction	
Plant (part) <sup>a)</sup>	A <sup>b)</sup>	R <sup>c)</sup>	Ts <sup>d)</sup>	I c)	T <sub>50</sub> f)	Hypocotyl <sup>g)</sup>	Radicle <sup>g)</sup>	ratio <sup>h)</sup>	
Chenopodiaceae									
Chenopodium album (L)	98	43	1.0	44	1.9	90	48	10	
Chenopodium album (R)	92	76	1.1	66	1.1	88	48	25	
Beta vulgaris (S)	96	86	1.5	56	1.2	109	64	5	
Beta vulgaris (R)**	90	75	4.3	16	2.1	57	21	25	
Spinacia oleracea (L)	94	68	2.4	28	1.7	119	38	5	
Spinacia oleracea (R)*	97	73	4.5	16	2.1	102	36	10	
Poligonaceae									
Poligonum blumei (S)**	84	48	1.3	31	1.5	86	37	25	
Fagopyrum esculentum (S)	100	235	2.4	100	1.0	107	60	12.5	
Labiatae									
Lamium amplexicaule (W)*	85	54	2.4	19	2.0	70	45	10	
Mentha spicata (L)*	99	51	1.9	27	1.9	121	28	8	
Mentha spicata (R)	95	75	0.9	80	1.2	139	89	8	
Salvia officinalis (L)	94	106	3.3	31	1.3	112	67	10	
Salvia officinalis (R)	98	86	3.1	27	1.9	123	83	8	
Melissa officinalis (L)**	39	23	3.7	3	2.3	101	57	8	
Melissa officinalis (R)	98	73	1.6	45	1.4	164	103	8	
Solanaceae	500-	0.005	07470	0.0250	2-01202	2.47632	7257679		
Solanum carolinense (S)	96	120	0.8	153	0.9	144	117	6	
Solanum melongena (S)	86	83	4.9	15	1.9	125	51	10	
Solanum melongena (B)	98	84	2.9	29	1.6	130	58	10	
I vconersicon esculentum (S)	96	136	5.9	23	1.9	135	37	10	
Lycopersicon esculantum (B)	98	123	33	38	1.4	131	45	10	
Solonum tuberosum (I.)	99	75	1.3	127	13	127	62	6	
Solanum Tuberosum (L)	99	72	0.4	167	0.8	148	88	2.5	
Cucurbitaceae	"	12	0.4	107	0.0	140	00	2.2	
Cucumic satime (S)	00	123	31	41	13	187	78	5	
Cucumis sativus (S)	08	224	4 3	52	1.3	159	71	10	
Cucumis salivas (K)	90	153	4.5	30	1.8	110	50	12.5	
Cucurbita maxima (S)	100	100	2.2	19	1.0	112	84	17	
Citrullus Investus (I.)	100	109	2.5	40	1.2	113	60	6	
Citrulius Ianatus (L)	95	102	3.1	20	1.5	133	50	6	
Citrulius Ianatus (Stem)	90	102	3.0	22	2.7	112	74	12.5	
Citruitus Ianatus (K)	94	103	4.2	25	2.2	115	74	12.5	
Other genus	07	60		51	1.4	00	67	6	
Stellaria media (W)	97	09	1.4	21	1.4	99	07	10	
Cerastium glomeratum (W)	90	14	2.1	31	1.7	103	29	10	
Houltuynia cordata (S)	98	33	3.0	9	3.4	62	20	5	
Houttuynia cordata (R)	95	66	0.9	68	1.5	126	50	10	
Garium spurium (W) *	92	65	2.1	29	1.8	85	58	10	
Paederia scandens (L)	97	46	1.5	86	1.2	123	92	12.5	
Paederia scandens (Stem)	98	52	0.5	96	1.1	143	98	10	
Portulaca oleracea (W)	90	117	4.8	22	1.9	119	49	3	
Calystegia hederacea (S)	96	66	2.4	27	1.9	94	60	10	
Calystegia hederacea (R)	99	87	2.5	35	1.8	103	46	10	
Commelina communis (L)	91	62	4.5	12	1.8	132	65	10	
Phytolacca americana (L) **	98	44	2.3	19	2.2	57	33	6	
Phytolacca americana (Stem)	93	61	1.6	37	1.5	124	39	6	
Phytolacca americana (R) ***	75	40	1.8	16	1.8	78	37	10	
Plantago major (L)	88	101	3.5	26	1.6	121	73	5	
Plantago major (R)	84	75	3.3	19	1.8	138	74	12.5	
Oenothera biennis (S)	84	48	1.3	31	1.5	105	39	25	

#### (Table 1, continued)

	Germination test					Growth test		Extraction	
Plant (part) <sup>a)</sup>	A <sup>b)</sup>	R <sup>c)</sup>	Ts <sup>d)</sup>	I c)	T50 <sup>0</sup>	Hypocotyl <sup>g)</sup>	Radicle <sup>g)</sup>	ratio <sup>h)</sup>	
Oenothera biennis (R)	91	61	1.1	52	1.2	119	40	25	
Amaranthus tricolor (L)	92	66	4.0	15	2.4	93	81	6	
Amaranthus tricolor (Stem)	94	100	4.0	23	2.1	116	97	10	
Impatiens balsamina (L)	93	101	3.3	28	1.9	117	64	6	
Impatiens balsamina (Stem)	93	80	3.1	24	1.9	136	77	3	
Paulowinia tomentosa (L)	100	53	1.2	45	1.5	119	61	12.5	
Paulowinia tomentosa (Stem)	100	139	1.5	98	1.2	136	52	12.5	
Colocasia esculenta (L) ***	92	22	6.3	3	4.9	22	32	10	
Colocasia esculenta (Stem) *	99	74	3.1	24	1.8	133	35	5	
Colocasia esculenta (R)	98	95	4.9	20	1.9	149	42	10	
Brassica napus (S)	84	85	1.3	56	1.2	108	98	10	
Brassica napus (R) **	76	60	1.3	37	1.3	98	37	10	
Brassica juncea (S)	87	61	1.6	34	1.5	154	71	3	
Brassica campestris var. perviridis (L)	93	27	0.5	58	1.6	141	94	3	
Brassica oleracea var. capitata (L) *	76	97	5.6	14	1.4	146	88	5	
Average	92.3	78.7	2.4	47.0	1.71	112.6	58.4		
Standard deviation $(\sigma_{n-1})$	9.4	40.3	1.5	38.2	0.72	26.8	21.6		

a): S: Shoot, R: Root, W: Whole plant (=S+R), L: Leaf.

b): Germination percentage at the end of germination process estimated with cumulative germination curves fitted to Richards' function (% of control).

c): Germination rate (% of germinated seeds per day, % of control).

d): Start of germination (a time spent until one seed germinates, ratio of control).

e): Germination index  $(I = A \cdot R/Ts)$ .

f): 50% germination time (a time spent until 50% of seeds can germinate, ratio of control).

g): % of control (control dish is cultured with water).

h): Extraction ratio (mg-D.W./m/). Extraction ratio was determined in order that EC of the assay solution did not exceed 1 mS/cm.

Plant name with an underline denotes strong inhibition in one of the following parameters: hypocotyl elongation, radicle elongation, A (germination %), 1 (germination index).

\*, \*\* and \*\*\* show the plant name together with its degree of inhibition. When the values exceed the criteria of average  $\pm \sigma$ , there would be a possibility of inhibition.

Condition	Cultivated plant	Plant height (%)	Shoot D.W. (%)	Root D.W. (%)
60°C-oven	Oryza sativa (upland)	101	71	83
dried leaf	Zea mays	110	104	103
	Solghum bicolor	91	77	91
	Glycine max	98	97	107
	Phaseolus vulgaris	160	101	88
	Arachis hypogaea	105	95	134
	Solanum melongena	86	91	95
	Cucumis sativus	82	83	102
Fresh leaf	Zea mays	85	88	69
	Phaseolus vulgaris	32	27	25
	Cucumis sativus	96	86	57

Table 2.	Plant	growth after	the	incorporation	of	velvetbean	leaves	to	soi	ls
----------	-------	--------------	-----	---------------	----	------------	--------	----	-----	----

Crop	Treatment <sup>a)</sup>	Weed population (g D.W./m <sup>2</sup> )	Weed species observed <sup>b)</sup> (April 14, 1988)
Upland rice	3yr.c	5.11 (49.4) <sup>c)</sup>	13567891011
Eggplant	3yr.c	16.82 (40.1)	123567891011121314
Tomato	3yr.c	4.92 (64.9)	15691111
Velvetbean	2yr.c	0.00 ( 0.0)	No emergence
Velvetbean	lyr.c, lyr.f	3.05 (74.8)	0.1012131618
Fallow	3yr.f	0.97 (37.3)	1261012131516

Table 3. Weed population in continuous cropping fields

a): 3yr.c; Continuous cropping for 3 years, 1yr.c, 1yr.f; Cultivated for 1 year, followed by fallow next year (test year), 3yr.f; Fallow for 3 years without fertilizer.

b): Species appeared in each plot: 1) sticky chickweed (Cerastium glomeratum), 2 miminagusa (Cerastium vulgatum var. augustifolium), 3 annual fleabane (Erigeron annuus), 4 Philadelphia fleabane (Erigeron philadelphicus), 5 starwort (Stellaria alsine var. undulata) 6 floating foxtail (Alopecurus geniculatus), 7 narrowleaf vetch (Vicia angustifolia), 8 flexuosa bittercress (Cardamine flexuosa), 9 inugarashi (Rorippa atrovirens), 10 common dandelion (Taraxacum officinale), 11 Japanese mugwort (Artemisia princeps), 12 Canadian fleabane (Erigeron canadensis), 13 hahakogusa (Gnaphalium affine), 14 blady grass (Imperata cylindrica), 15 meadowgrass (Poa annua), 16 creeping wood-sorrel (Oxalis corniculata), 17 shepherd's-purse (Capsella bursa-pastoris), 18 prickly sowthistle (Sonchus asper).

c): Numbers in parenthesis are percentages of chickweed, a dominant species. Source: Fujii et al. (1991)<sup>8)</sup>.

Receiver plant	Donor plant	Leaf area (cm <sup>2</sup> )	Shoot D.W. (g)	Root D.W. (g)
Lettuce	Lettuce	30.4 (89)	53.9 (96)	12.0 (101)
	Velvetbean	21.5 (63)	39.3 (70)	5.7 (48)
	None	34.2 (100)	56.3 (100)	11.9 (100)
Kidney bean	Kidney bean	87.9 (97)	343 ( 96)	148 ( 79)
1/2/19/2010 17 10 17 8 82/2010 11	Velvetbean	81.4 ( 90)	344 (96)	153 (81)
	None	90.3 (100)	358 (100)	188 (100)

Table 4. Effect of mixed culture of velvetbean to the growth of lettuce and kidney bean under a stairstep experiment

Underline shows strong inhibition.

Numbers in the parentheses are percentages of control. Source: Fujii et al. (1991)<sup>13)</sup>.

This effect ran out two weeks after the incorporation. Dried leaves incorporation showed no inhibition.

(3) Weed prevalence in the fields of velvetbean stands

Table 3 shows weed populations in spring in the continuous cropping fields. The velvetbean plot showed a lower population of weeds dominated by sticky chickweed (*Cerastium glomeratum*) than the other plots of eggplant, tomato, upland rice and fallow did.

(4) Mixed culture of velvetbean with stairstep apparatus The stairstep method is a sort of sand culture with a nutrient-solution recirculating system on a staircase bed. Through this method, the presence of velvetbean reduced the growth of lettuce shoot growth to the level of 70% of the control (Table 4). This result indicates that velvetbean root exudates have allelopathic activity.

(5) Allelopathic compound in velvetbean

The analysis on effective compound of velvetbean in restraining the growth of companion plants confirmed its association with L-DOPA (Figs. 1, 2 and 3). It is well known that velvetbean seeds contain a high concentration of L-DOPA  $(6-9\%)^{4,25}$ , which



Fig. 1. HPLC analysis of L-DOPA and dopamine in the leaf and root of velvetbean

plays a role of chemical barrier to insect attacks<sup>1,22)</sup>. In mammalian brain, L-DOPA is the precursor of dopamine, a neurotransmitter, and also important intermediates of alkaroids. In animal skin, hair, feathers, fur and insect cuticle, L-DOPA is oxidized through dopaquinone to produce melanin. As L-DOPA is an intermediate and rapidly metabolized, normal tissues have little concentrations of L-DOPA.

Fresh velvetbean leaves contain as much as 1% of L-DOPA. It actually exudes from root, and its concentration reaches 1 ppm in water-culture solution, and 50 ppm in the vicinity of roots. This concentration is high enough to reduce the growth







Fig. 3. Effects of velvetbean (Mucuna) leaf extract and L-DOPA on the radicle growth of lettuce Source: Fujii et al. (1991)<sup>11)</sup>.

of neighboring plants and the growth inhibition in a mixed culture is shown in agar-medium culture<sup>6,7)</sup>. It also leaches out from leaves with rain drops or fog dew. Since velvetbean produces 20-30 t of fresh leaves and stems per ha, approximately 200-300 kg of L-DOPA may be added to soils a year. (6) Phytotoxic effects of L-DOPA

Some effects of L-DOPA on germination and growth of the selected crops and weeds are summerized in Table 5. L-DOPA suppresses the radicle

244

growth of lettuce and chickweed to the level of 50% of the control at 50 ppm  $(2 \times 10^{-4} \text{ mol/l})$ . It is however less effective to the hypocotyl growth and practically not effective to the germination (Fig. 2). L-DOPA strongly inhibits the plant growth of *Cerastium glomeratum, Spergula arvensis* (both Caryophyllaceae), *Linum usitatissimum* and *Lactuca sativa*, and moderately inhibits the growth of Compositae, while very limited effects on Gramineae and Leguminosae. Such selective effectiveness is comparable with other candidates of allelochemicals<sup>3,5)</sup>. L-DOPA contained in fresh velvetbean leaves fully attributes to the plant growth inhibition through

Table 5. Effect of L-DOPA on the growth of radicle of some weeds

Scientific name (family) <sup>a)</sup>	EC50 (mM) <sup>b)</sup>	English name
Cerastium glomeratum (ck)	0.10	Sticky chickweed
Spergula arvensis (ck)	0.20	Corn spurrey
Linum usitatissimum (ln)	0.20	Flax
Lactuca sativa (co)	0.20	Lettuce
Solidago altissima (co)	0.46	Tall golden-rod
Taraxacum officinale (co)	1.30	Common dandelion
Amaranthus lividus (am)	0.76	Wild blite
Miscanthus sinensis (gr)	0.86	Chinese fairygrass
Eleusine coracana (gr)	1.00	African millet
Setaria faberi (gr)	1.60	Giant foxtail
Plantago asiatica (pl)	1.40	Asiatic plantain
Trifolium pratense (le)	2.00	Red clover
Vicia villosa (le)	2.00	Hairly vetch

 a): ck; Caryophyllaceae, ln; Linaceae, co; Compositae, am; Amaranthaceae, gr; Gramineae, pl; Plantaginaceae, le; Leguminosae.

b): 50% inhibition concentration. Source: Fujii et al. (1991)<sup>11)</sup>. its crude extract (Fig. 3). The result that L-DOPA strongly suppresses the growth of chickweed agrees with weed inhibition in the velvetbean field (Table 3). All these data suggest that L-DOPA function as an allelopathic chemical.

In the aged leaves, the content of dopamine increases (Fig. 1), and L-DOPA and dopamine are presumably changed to catechol in the litter. Table 6 shows activities of these compounds assayed.

It is an earlier understanding that velvetbean smothers weeds under its rapid and thick covering effect with leaves. However, the above-noted results suggest that L-DOPA or its associate compounds, accumulated in an extremely high concentration in plants, function as an allelochemical in reducing a weed population. The role of L-DOPA in velvetbean seeds was earlier regarded as a chemical barrier to insect attacks<sup>1)</sup>. It is now confirmed however that it plays another role of its allelopathic activity in weed control.

Apart from the L-DOPA in velvetbean, caffeine in a coffee tree<sup>27)</sup>, mimosine in *Lucaena* spp.<sup>3)</sup>, nordihydroguaiaretic acid (NDGA) in a creosote bush<sup>5)</sup>, each of which is contained in a high quantity in the respective plants, are well known to have physiological effects on animals, while their associations with other plants in terms of allelopathy have only recently been identified. It is expected that some secondary metabolites would be identified in the field of allelopathy.

Since velvetbean has special abilities such as weed smothering<sup>8,11)</sup>, tolerance to pests<sup>1,18)</sup>, suppression of nematode population<sup>24,30,31)</sup>, and soil improvement in its physical structure<sup>18)</sup>, it could be more widely used in future to reduce applications of

Diant name	Colontific nome	Compounds						
Fiant name	Scientific name	L-DOPA	Dopamine	Pyrocatechol				
Lettuce	Lactuca sativa	0.20	6.3	0.73				
Tall goldenrod	Solidago altissima	0.46	>3.2	0.36				
Common dandelion	Taraxacum officinale	1.30	1.6	0.73				
Wild blite	Amaranthus lividus	0.76	>3.2	< 0.27				
Chinese fairygrass	Miscanthus sinensis	0.86	>3.2	0.73				
Giant foxtail	Setaria faberi	2.00	4.4	2.70				
Sticky chickweed	Cerastium glomeratum	0.10	>3.2	0.55				
Corn spurrey	Spergula arvensis	0.20	1.6	1.40				

Table 6. Effects of L-DOPA and related compounds in velvetbean on the growth of radicles of lettuce and some weeds

50% inhibition concentration.

artificial chemicals to a lower level. Velvetbean seed yields are very high in the tropics, and the seed contains a high level of protein with a useful protein score. If detrimental factors such as L-DOPA and trypsine inhibitors could be eliminated through proper cooking<sup>23)</sup>, it would also contribute to alleviation of the food problems in some tropical countries.

#### References

- Bell, E. A. & Janzen, D. H. (1971): Medical and ecological considerations of L-DOPA and 5-HTP in seeds. *Nature*, 229, 136-137.
- Bell, D. T. & Koeppe, D. E. (1972): Noncompetitive effects of giant foxtail on the growth of corn. Agron. J., 64, 321-325.
- Chou, C-H. & Kuo, Y-L. (1986): Allelopathic research of subtropical vegetation in Taiwan. III. Allelopathic exclusion of understory by *Leucaena leucocephala* (Lam.) de Wit. J. Chem. Ecol., 12, 1431–1448.
- Damodaran, M. & Ramaswamy, R. (1937): Isolation of L-3,4-dihydroxyphenylalanine from the seeds of Mucuna pruriens. Biochem. J., 31, 2149-2152.
- Elacovitch, S. D. & Stevens, K. L. (1985): Phytotoxic properties of nordihydroguaiaretic acid; a lignan from *Larrea tridentata* (Creosote bush). J. Chem. Ecol., 11, 27-33.
- 6) Fujii, Y. & Shibuya, T. (1991): A new bioassay for allelopathy with agar medium. I. Assessment of allelopathy from litter leacheate by sandwich method. *Weed Res. Jpn.*, 36 (suppl.), 150-151 [In Japanese].
- Fujii Y. & Shibuya, T. (1991): A new bioassay for allelopathy with agar medium. II. Mixed culture of allelopathic candidates with acceptor plants in agar medium. Weed Res. Jpn., 36 (suppl.), 152-153 [In Japanese].
- Fujii, Y., Shibuya, T. & Usami, Y. (1991): Allelopathic effect of *Mucuna pruriens* on the appearance of weeds. *Weed Res. Jpn.*, 36, 43-49 [In Japanese with English summary].
- Fujii, Y., Shibuya, T. & Yasuda, T. (1990): Method for screening allelopathic activities by using the logistic function (Richards' function) fitted to lettuce seed germination and growth curves. Weed Res. Jpn., 35, 353-361 [In Japanese with English summary].
- 10) Fujii, Y., Shibuya, T. & Yasuda, T. (1990): Survey of Japanese weeds and crops for the detection of waterextractable allelopathic chemicals using Richards' function fitted to lettuce germination test. Weed Res. Jpn., 35, 362-370 [In Japanese with English summary].
- Fujii, Y., Shibuya, T. & Yasuda, T. (1991): L-3, 4-Dihydroxyphenylalanine as an allelochemical candidate from *Mucuna pruriens* (L.) DC. var. *utilis. Agr. Biol. Chem.*, 55, 617-618.

- 12) Fujii, Y., Shibuya, T. & Yasuda, T. (1991): Discrimination of allelopathy of tomato plant by stairstep experiment and rotary greenhouse experiment. *Jpn. J. Soil Sci. Plant Nutr.*, 62, 150-155 [In Japanese with English summary].
- 13) Fujii, Y., Shibuya, T. & Yasuda, T. (1991): Discrimination of allelopathy of velvetbean (*Mucuna pruriens*) with stairstep experiment and rotary greenhouse experiments. *Jpn. J. Soil Sci. Plant Nutr.*, 62, 258–264 [In Japanese with English summary].
- 14) Fujii, Y., Shibuya, T. & Yasuda. T., (1991): Discrimination of allelopathy of upland rice, taro, and oat by substitutive experiment and its modified experiments. *Jpn. J. Soil Sci. Plant Nutr.*, 62, 257-362 [In Japanese with English summary].
- 15) Fujii, Y., Shibuya, T. & Yasuda, T. (1991): Intercropping of velvetbean (*Mucuna pruriens*) by substitutive experiments: suggestion of companion plants with corn and kidney bean. Jpn. J. Soil Sci. Plant Nutr., 62, 363-370 [In Japanese with English summary].
- 16) Fujii, Y. et al. (1991): Survey of Japanese medicinal plants for the detection of allelopathic properties. *Weed Res. Jpn.*, 36, 36-42 [In Japanese with English summary].
- 17) Goda, Y., Shibuya, M. & Sankawa, U. (1987): Inhibitors of prostaglandin biosynthesis from *Mucuna bird*woodiana. Chem. Pharm. Bull., 35, 2675-2677.
- 18) Hulugalle, N. R., Lal, R. & Terkuile, C. H. H. (1986): Amelioration of soil physical properties by *Mucuna* after mechanized land clearing of a tropical rain forest. *Soil Science*, 141, 219–224.
- 19) Lehle, F. R. & Putnam, A. R. (1982): Quantification of allelopathic potential of sorghum residues by novel indexing of Richards' function fitted to cumulative cress seed germination curves. *Plant Physiol.*, 69, 1212– 1216.
- 20) Lorenzi, H. (1984): Consideracoes sobre plantas daninhas no plantio direto. *In* Plant Direto no Brasil. eds. Torrado, P. V. & Aloisi, R. R., Fundacao Cargill, Campinas, 24-35.
- Miller, E. R. (1920): Dihydroxyphenylalanine, a constitute of the velvetbean. J. Biol. Chem., 44, 481-486.
- 22) Premchand (1981): Presence of feeding deterrent in velvetbean (Mucuna cochinchinensis). Indian J. Entomol., 43, 217-219.
- 23) Ravindran G. & Ravindran, G. (1988): Nutritional and anti-nutritional characteristics of mucuna (*Mucuna* utilis) bean seeds. J. Sci. Food Agr., 46, 71-79.
- 24) Reddy, K. C. et al. (1986): Tropical legumes for green manure. II. Nematode populations and their effects on succeeding crop yields. Agron. J., 78, 5-10.
- 25) Rehr, S. S., Janzen, D. H. & Feeny, P. P. (1973): L-Dopa in legume seeds: a chemical barrier to insect attack. Science, 181, 81-82.
- 26) Richards, F. J. (1959): A flexible growth function for emprical use. J. Exp. Bot., 10, 290-300.
- 27) Rizvi, S. J. H., Mukerji, D. & Mathur, S. N. (1981): Selective phytotoxicity of 1,3,7-trimethylxanthine

between Phaseolus mungo and some weeds. Agr. Biol. Chem., 45, 1255-1256.

- 28) Taib, I. M., Sin, L. & Alif A. F. (1979): Chemical weed control in legume management. *In Proceedings* of the rubber research institute of Malaysia planters' conference, 1979. 375-391.
- 29) Tateishi, Y. & Ohashi, H. (1981): Eastern Asiatic species of Mucuna (Leguminosae). Bot. Mag., 94, 91-105.
- 30) Tenente, R. C. V. & Lordello, L. G. E. (1980): Influence of *Stizolobium aterrimum* on the life-cycle of *Meloidogyne incognita*. *In* Sociedade Brasileira de Nematologia, 1980. 213–215.
- 31) Tenente, R. C. V., Lordello, L. G. E. & Dias J. F. S. (1982): A study of the effect of root exudates of *Stizolobium aterrimum* on the hatching, penetration and

development of *Meloidogyne incognita* race 4. In Sociedade Brasileira de Nematologia, 1982. 271-284.

- 32) Watt, J. M. & Breyer-Brandwijk, M. G. (1962): Medicinal and poisonous plants of southern and eastern Africa. (2nd ed.) E. & S. Livingstone, Edinburgh and London, 631-634.
- Wilmot-Dear, C. M. (1983): A revision of Mucuna (Leguminosae-Phaseolae) in China and Japan. Kew Bull., 39, 23-65.
- 34) Yasuda, T., Shibuya, T. & Fujii, Y. (1991): Discrimination of allelopathy of common lambsquarters by stairstep experiments. *Jpn. J. Soil Sci. Plant Nutr.*, 62, 252-257 [In Japanese with English summary].

(Received for publication, Sept. 27, 1991)