

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

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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 pruriens* 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 seedling 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 alkaloids. The present studies revealed that L-DOPA contained in a large amount (about 1% of the fresh weight in leaves and roots of velvetbean) 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*)^{20,28)}. 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 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^{8,15)}.

The genus *Mucuna* consists of 100-200 species growing in the tropics and subtropics^{29,33)}. 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 China³²⁾. 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^{9,10,16}

Approximately 70 plant species were tested on their allelopathy with a Richards' function method²⁶, which proved to be suited to germination tests of lettuce and some weed plants¹⁹. 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)¹⁹. 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 stands⁸

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^{12,13,34} and substitutive experiments^{14,15,17}. The stairstep experiment was designed according to the method of Bell and Koeppel². The substitutive experiment was modified from the methods of the relevant references^{14,15,17}.

(6) Isolation and identification of allelopathic substances¹¹

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).

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

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

(continue)

(Table 1, continued)

Plant (part) ^{a)}	Germination test					Growth test		Extraction ratio ^{b)}
	A ^{b)}	R ^{c)}	T _s ^{d)}	I ^{e)}	T ₅₀ ^{f)}	Hypocotyl ^{g)}	Radicle ^{g)}	
Chenopodiaceae								
<i>Chenopodium album</i> (L)	98	43	1.0	44	1.9	90	48	10
<i>Chenopodium album</i> (R)	92	76	1.1	66	1.1	88	48	25
<i>Beta vulgaris</i> (S)	96	86	1.5	56	1.2	109	64	5
<i>Beta vulgaris</i> (R)**	90	75	4.3	16	2.1	57	21	25
<i>Spinacia oleracea</i> (L)	94	68	2.4	28	1.7	119	38	5
<i>Spinacia oleracea</i> (R)*	97	73	4.5	16	2.1	102	36	10
Poligonaceae								
<i>Poligonum blumei</i> (S)**	84	48	1.3	31	1.5	86	37	25
<i>Fagopyrum esculentum</i> (S)	100	235	2.4	100	1.0	107	60	12.5
Labiatae								
<i>Lamium amplexicaule</i> (W)*	85	54	2.4	19	2.0	70	45	10
<i>Mentha spicata</i> (L)*	99	51	1.9	27	1.9	121	28	8
<i>Mentha spicata</i> (R)	95	75	0.9	80	1.2	139	89	8
<i>Salvia officinalis</i> (L)	94	106	3.3	31	1.3	112	67	10
<i>Salvia officinalis</i> (R)	98	86	3.1	27	1.9	123	83	8
<i>Melissa officinalis</i> (L)**	39	23	3.7	3	2.3	101	57	8
<i>Melissa officinalis</i> (R)	98	73	1.6	45	1.4	164	103	8
Solanaceae								
<i>Solanum carolinense</i> (S)	96	120	0.8	153	0.9	144	117	6
<i>Solanum melongena</i> (S)	86	83	4.9	15	1.9	125	51	10
<i>Solanum melongena</i> (R)	98	84	2.9	29	1.6	130	58	10
<i>Lycopersicon esculentum</i> (S)	96	136	5.9	23	1.9	135	37	10
<i>Lycopersicon esculentum</i> (R)	98	123	3.3	38	1.4	131	45	10
<i>Solanum tuberosum</i> (L)	99	75	1.3	127	1.3	127	62	6
<i>Solanum tuberosum</i> (Stem)	99	72	0.4	167	0.8	148	88	2.5
Cucurbitaceae								
<i>Cucumis sativus</i> (S)	99	123	3.1	41	1.3	187	78	5
<i>Cucumis sativus</i> (R)	98	224	4.3	52	1.3	159	71	10
<i>Cucurbita maxima</i> (S)	93	153	4.8	30	1.8	119	50	12.5
<i>Cucurbita maxima</i> (R)	100	109	2.3	48	1.1	113	84	17
<i>Citrullus lanatus</i> (L)	95	102	3.7	26	1.3	133	69	6
<i>Citrullus lanatus</i> (Stem)	96	116	3.0	36	1.7	129	59	6
<i>Citrullus lanatus</i> (R)	94	103	4.2	23	2.2	113	74	12.5
Other genus								
<i>Stellaria media</i> (W)	97	69	1.4	51	1.4	99	67	5
<i>Cerastium glomeratum</i> (W) *	90	74	2.1	31	1.7	103	29	10
<i>Houttuynia cordata</i> (S) ***	98	33	3.6	9	3.4	62	26	5
<i>Houttuynia cordata</i> (R)	95	66	0.9	68	1.5	126	50	10
<i>Garium spurium</i> (W) *	92	65	2.1	29	1.8	85	58	10
<i>Paederia scandens</i> (L)	97	46	1.5	86	1.2	123	92	12.5
<i>Paederia scandens</i> (Stem)	98	52	0.5	96	1.1	143	98	10
<i>Portulaca oleracea</i> (W)	90	117	4.8	22	1.9	119	49	3
<i>Calystegia hederacea</i> (S)	96	66	2.4	27	1.9	94	60	10
<i>Calystegia hederacea</i> (R)	99	87	2.5	35	1.8	103	46	10
<i>Commelina communis</i> (L)	91	62	4.5	12	1.8	132	65	10
<i>Phytolacca americana</i> (L) **	98	44	2.3	19	2.2	57	33	6
<i>Phytolacca americana</i> (Stem)	93	61	1.6	37	1.5	124	39	6
<i>Phytolacca americana</i> (R) ***	75	40	1.8	16	1.8	78	37	10
<i>Plantago major</i> (L)	88	101	3.5	26	1.6	121	73	5
<i>Plantago major</i> (R)	84	75	3.3	19	1.8	138	74	12.5
<i>Oenothera biennis</i> (S)	84	48	1.3	31	1.5	105	39	25

(continue)

(Table 1, continued)

Plant (part) ^{a)}	Germination test					Growth test		Extraction ratio ^{h)}
	A ^{b)}	R ^{c)}	T _s ^{d)}	I ^{e)}	T ₅₀ ^{f)}	Hypocotyl ^{g)}	Radicle ^{g)}	
<i>Oenothera biennis</i> (R)	91	61	1.1	52	1.2	119	40	25
<i>Amaranthus tricolor</i> (L)	92	66	<u>4.0</u>	15	<u>2.4</u>	93	81	6
<i>Amaranthus tricolor</i> (Stem)	94	100	<u>4.0</u>	23	2.1	116	97	10
<i>Impatiens balsamina</i> (L)	93	101	3.3	28	1.9	117	64	6
<i>Impatiens balsamina</i> (Stem)	93	80	3.1	24	1.9	136	77	3
<i>Paulownia tomentosa</i> (L)	100	53	1.2	45	1.5	119	61	12.5
<i>Paulownia tomentosa</i> (Stem)	100	139	1.5	98	1.2	136	52	12.5
<i>Colocasia esculenta</i> (L) ***	92	<u>22</u>	<u>6.3</u>	<u>3</u>	<u>4.9</u>	<u>22</u>	<u>32</u>	10
<i>Colocasia esculenta</i> (Stem) *	99	74	3.1	24	1.8	133	<u>35</u>	5
<i>Colocasia esculenta</i> (R)	98	95	4.9	20	1.9	149	42	10
<i>Brassica napus</i> (S)	84	85	1.3	56	1.2	108	98	10
<i>Brassica napus</i> (R) **	<u>76</u>	60	1.3	37	1.3	98	<u>37</u>	10
<i>Brassica juncea</i> (S)	87	61	1.6	34	1.5	154	71	3
<i>Brassica campestris</i> var. <i>perviridis</i> (L)	93	27	0.5	58	1.6	141	94	3
<i>Brassica oleracea</i> var. <i>capitata</i> (L) *	<u>76</u>	97	<u>5.6</u>	14	1.4	146	88	5
Average	92.3	78.7	2.4	47.0	1.71	112.6	58.4	
Standard deviation (σ_{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 ($I = A \cdot R / T_s$).

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./ml). 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 %), I (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.

Table 2. Plant growth after the incorporation of velvetbean leaves to soils

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

Table 3. Weed population in continuous cropping fields

Crop	Treatment ^{a)}	Weed population (g D.W./m ²)	Weed species observed ^{b)} (April 14, 1988)
Upland rice	3yr.c	5.11 (49.4) ^{c)}	①③⑤⑥⑦⑧⑨⑩⑪
Eggplant	3yr.c	16.82 (40.1)	①②③⑤⑥⑦⑧⑨⑩⑪⑫⑬⑭
Tomato	3yr.c	4.92 (64.9)	①⑤⑥⑨⑫⑬⑰
Velvetbean	2yr.c	0.00 (0.0)	No emergence
Velvetbean	1yr.c, 1yr.f	3.05 (74.8)	①⑩⑫⑬⑮⑰
Fallow	3yr.f	0.97 (37.3)	①②⑥⑩⑫⑬⑮⑰

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. *angustifolium*), 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)⁸⁾.

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

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

Underline shows strong inhibition.

Numbers in the parentheses are percentages of control.

Source: Fujii et al. (1991)¹³⁾.

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 staircase apparatus

The staircase 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

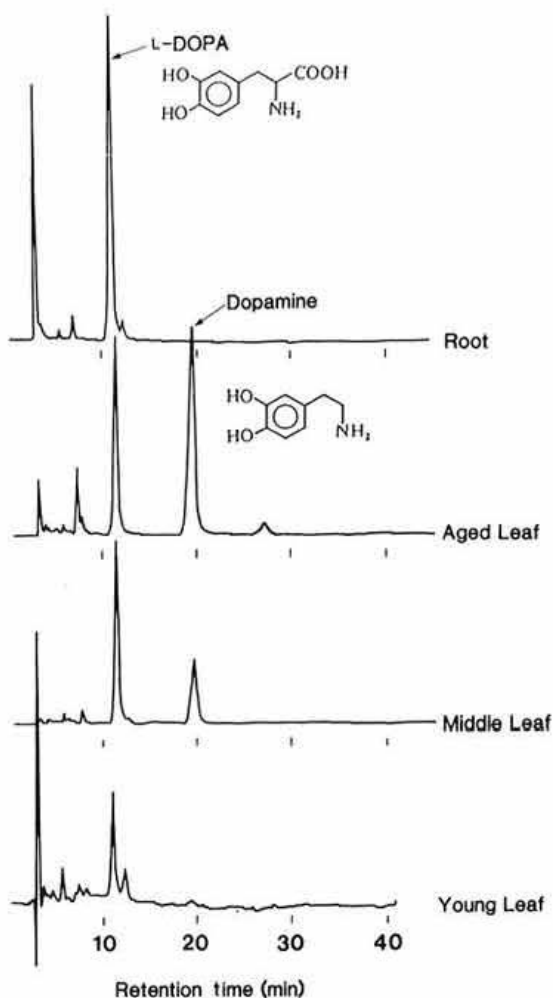


Fig. 1. HPLC analysis of L-DOPA and dopamine in the leaf and root of velvetbean

plays a role of chemical barrier to insect attacks^{1,22}. In mammalian brain, L-DOPA is the precursor of dopamine, a neurotransmitter, and also important intermediates of alkaloids. 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

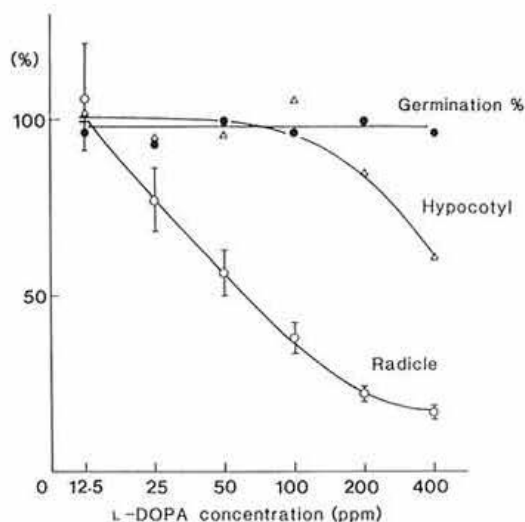


Fig. 2. Effects of L-DOPA on germination and growth of lettuce

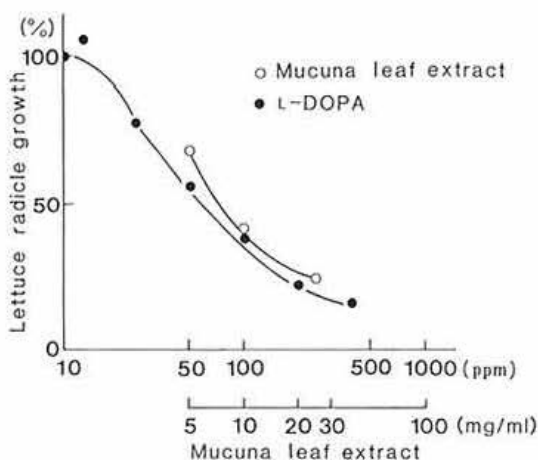


Fig. 3. Effects of velvetbean (*Mucuna*) leaf extract and L-DOPA on the radicle growth of lettuce Source: Fujii et al. (1991)¹¹.

of neighboring plants and the growth inhibition in a mixed culture is shown in agar-medium culture^{6,7}. 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 summarized in Table 5. L-DOPA suppresses the radicle

growth of lettuce and chickweed to the level of 50% of the control at 50 ppm (2×10^{-4} 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^{3,5}. 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) ^{a)}	EC ₅₀ (mM) ^{b)}	English name
<i>Cerastium glomeratum</i> (ck)	0.10	Sticky chickweed
<i>Spergula arvensis</i> (ck)	0.20	Corn spurrey
<i>Linum usitatissimum</i> (ln)	0.20	Flax
<i>Lactuca sativa</i> (co)	0.20	Lettuce
<i>Solidago altissima</i> (co)	0.46	Tall golden-rod
<i>Taraxacum officinale</i> (co)	1.30	Common dandelion
<i>Amaranthus lividus</i> (am)	0.76	Wild blite
<i>Miscanthus sinensis</i> (gr)	0.86	Chinese fairygrass
<i>Eleusine coracana</i> (gr)	1.00	African millet
<i>Setaria faberi</i> (gr)	1.60	Giant foxtail
<i>Plantago asiatica</i> (pl)	1.40	Asiatic plantain
<i>Trifolium pratense</i> (le)	2.00	Red clover
<i>Vicia villosa</i> (le)	2.00	Hairy 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)¹¹.

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 attacks¹¹. 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 tree²⁷), mimosine in *Lucaena* spp.³⁾, nordihydroguaiaretic acid (NDGA) in a creosote bush⁵), 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^{8,11}), tolerance to pests^{1,18}), suppression of nematode population^{24,30,31}), and soil improvement in its physical structure¹⁸), it could be more widely used in future to reduce applications of

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

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

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 trypsin inhibitors could be eliminated through proper cooking²³, it would also contribute to alleviation of the food problems in some tropical countries.

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(Received for publication, Sept. 27, 1991)