J. Fac. Appl. Biol. Sci.,
Hiroshima Univ. (1991), **30** : 19～29

The Interspecific and Inter-parametric Differences of Forage Crops in Response to Seed Treatment with Aqueous Extracts of Animal-waste Composts

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Received April 25, 1991

Abstract Experiments were conducted to find the best assay method to evaluate the inhibitory effects of animal-waste composts on crop growth. The inhibitory effects of 14 kinds of animal-waste composts on seed germination and seedling growth of 7 forage crops (corn, sorghum, Italian ryegrass, African millet, red clover, alfalfa and soybean) and Komatsuna (*Brassica rapa* L) were tested under laboratory conditions. The parameters measured were seed germination rate and root length, shoot length, fresh weight and dry weight of 72 h old seedlings.

The inhibitory effects of animal-waste composts varied among the test plant species and parameters. It is difficult to evaluate the inhibitory effects of a given compost by using a given parameter of a single plant. These results indicate that toxic substances in composts and their activity differ among the crop plant species. The necessity to identify the causal substances and to clarify their mode of action in plant growth is also indicated.

INTRODUCTION

The animal-waste composts have long been considered as organic manures and currently are widely utilized in crop production (CIEC, 1987, OSADA *et al,* 1990, ANDO *et al,* 1990). One of the major constraints associated in their use is the inhibitory effects shown on plant growth and development. Strauch (1987) suggested of direct or indirect hazardous effects on plant and soil system with the application of heavy doses of animal effluents. VETTER (1987) reported that the fertilization with animal slurry often cause negative effects on plants. Recently MARAMBE *et al.* (1989), OSADA *et al.* (1990) and ISIRIMAH and OJANUGA (1990) alarmed of potential inhibitory effects of animal-waste composts on seed germination and seedling growth of crops.

Seed germination and seedling growth are well known as the most sensitive stages of plants to the changes in surrounding environment. Thus, knowledge on the inhibitory effects caused by the animal-waste composts towards these precarious stages of plant life will be of prime importance as this will result in weakened plant growth ultimately affecting the yield.

The limited research carried out on inhibitory effects of animal-waste composts have mainly used a single crop as the indicator plant of compost toxicity. However, we assumed that the response of crops to the different kinds of animal-waste composts may differ with the plant species.

This study was conducted to investigate the best assay method to evaluate the inhibitory effects of 14 samples of animal-waste composts on seed germination and the seedling growth of seven forage crops and Komatsuna (*Brassica rapa* L), the plant that is mainly employed in investigation of phytotoxicity of animal-waste composts in Japan.

MATERIALS AND METHODS

Recipient crop seeds

The recipient crops are presented in Table 1. In each species, seeds of uniform colour and size were selected for the experiments.

Table 1. The recipient crop species

Crop (cv)	Abbreviation
Corn: *Zea mays* L (RM 100)	C N
Sorghum: *Sorghum bicolor* Moench (Sweet sorgo)	S G
Italian ryegrass: *Lolium multiflorum* Lam Turf. (Waseyutaka)	I R
African millet: *Eleusine corocana* L Gaertn. (Yukijirushi kei)	A M
Red clover: *Trifolium pratense* L (Kenland)	R C
Alfalfa: *Medicago sativa* Pers (Du-puits)	A F
Soybean: *Glycine max* L Merr. (Kurosengoku)	S B
Komatsuna: *Brassica rapa* L (Osome)	K M

Preparation of compost extracts

Fourteen samples of animal-waste composts (6 samples of cattle-waste, 5 samples of chicken-waste and 3 samples of hog-waste) were collected at the time of their dispatch to the market from Western part of Japan. Table 2 presents the types of composts and some of their characteristics.

All the samples were oven dried at 60°C for 12 h for partial sterilization and stabilization of the samples. Then the dried composts were pulverized in a mill and were stored in plastic bottles until further use. The samples were extracted at 1 and 4% concentrations with deionized water after homogenizing for 1 h in a mechanical shaker. Then the extracts were centrifuged at 1600 g and filtered through a Toyo No. 6 filter paper. The filtrates were immediately used for the experiments with two replicates. Deionized water was used as the control.

Seed treatments and germination measurements

Seeds were imbibed in deionized water for 0, 1, 2, 4, 6, 8 and 10 h, at a rate of 1 g/3 ml water, to identify the imbibition duration that gives the optimal water uptake (fresh weight gain) of the seeds for early and better germination. The imbibed seeds were immediately blotted on a paper towel and the fresh weight was measured. The difference between the original and imbibed seed-fresh weight was considered as an estimate of the seed-water uptake.

The seeds imbibed in deionized water or compost extracts (imbibition duration was selected from the above experiment) were placed on two sheets of Toyo No. 3 filter paper

Table 2. The types of composts and some of their chemical characteristics.

Compost type and No.	MC[a)] %	pH	EC[b)] mS/cm	N %	P %	K %	Zn ppm.	Cu ppm.
				(Total-dry weight basis)				
Cattle-waste								
1	31.2	5.5	9.3	2.1	1.0	2.3	124	60
2	57.7	8.1	3.7	1.5	0.1	2.0	139	62
3	60.8	9.2	1.3	2.0	0.8	1.5	112	34
4	34.8	9.4	5.5	1.7	0.7	2.7	139	23
5	57.0	8.9	0.9	1.2	0.6	1.1	263	51
6	52.1	8.9	1.2	2.4	1.1	0.8	229	147
Chicken-waste								
7	38.9	9.2	5.5	2.4	4.4	2.0	619	69
8	12.9	8.1	7.7	3.7	2.0	2.7	269	86
9	17.6	9.0	7.0	3.7	2.3	2.0	441	50
10	16.7	8.5	5.9	5.2	1.8	2.4	302	65
11	0.1	12.8	21.6	0.5	11.3	12.2	1707	433
Hog-waste								
12	16.6	9.1	5.4	2.4	2.7	1.8	583	210
13	16.5	9.0	8.5	3.5	5.9	3.9	1333	282
14	13.5	9.1	5.1	3.8	2.7	1.8	611	308

a) moisture content and b) electrical conductivity were measured after extraction of 20 g compost DM/100 ml deionized water

treated with 3 ml of the respective sample solutions. Each Petri dish contained 50 seeds in the case of small-seeded crops (i. e. African millet, Italian ryegrass, red clover, alfalfa and Komatsuna) and 25 seeds in the case of large-seeded crops (i. e. corn, sorghum and soybean). The Petri dishes were incubated at 28°C under uninterrupted light condition (250 lx) for 72 h. Seeds with visible radicles were considered germinated.

Seedling growth

The root and shoot lengths, fresh weights and dry weights were recorded for 72 h old seedlings. Fresh weights were measured immediately after the seedlings were blotted on a paper towel. Dry weights were measured after 48 h oven drying at 65°C.

RESULTS

Water absorption of imbibed seeds

The results are presented in Figure 1. The leguminous crop seeds exhibited prolonged water uptake while the cereal crop seeds were approaching a plateau at a shorter time. The preliminary experiments conducted after different imbibition treatments explained that 4 h imbibition of crop seeds in an aqueous medium is optimum for early and better germination (Buddhi Marambe—unpublished data).

Seed germination

Table 3 presents the seed germination results of the test plant species in response to the 1% extracts of the 14 compost samples, in relation to the time. All the test plant species showed delayed radicle emergence when treated with the compost extracts when compared to the water control. However, the germination-inhibitory effects of composts

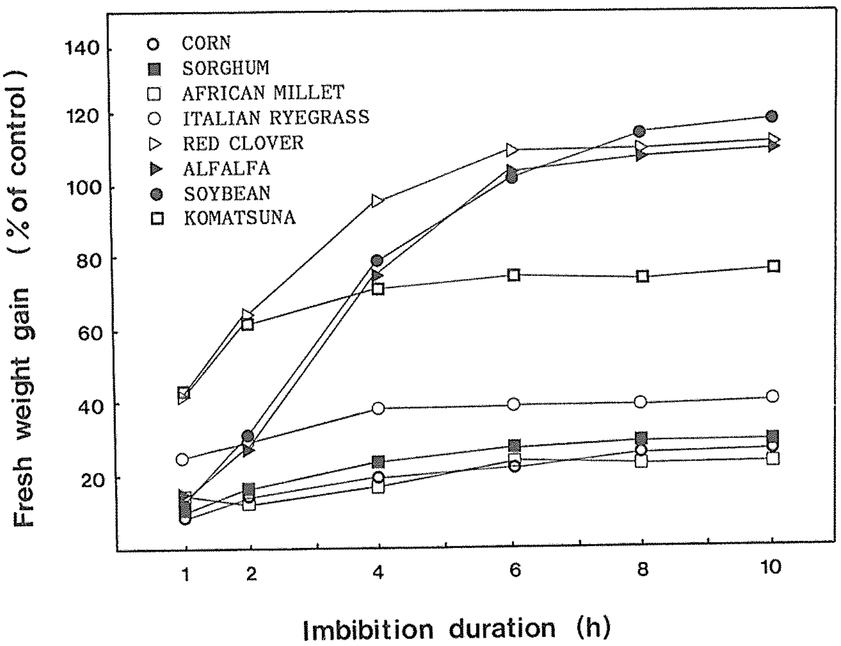

Fig. 1. Fresh weight gain of the forage crop seeds at different imbibition durations in deionized water.

varied with the crop plant species. Red clover showed the highest reduction in seed germination shown by a test plant species when treated with any of the compost extracts (32% reduction compared to the water control when treated with compost No. 10, a chicken-waste). Based on the mean germination results, red clover was the most sensitive to the compost treatments while soybean was the least affected.

Seedling growth

Tables 4 and 5 show the shoot length and root length respectively, of the 72 h old seedlings after the seed treatment with 1% extracts. All the crops, except alfalfa, showed the highest inhibition of shoot length when treated with compost No. 10. The root length of crops showed a different pattern of response to that of shoot length, where the individual species showed varied sensitivity to different compost treatments. Based on the mean shoot length results, alfalfa seems to be the most sensitive to the inhibitory action of animal-waste composts. The mean root length data indicate that Italian ryegrass is the most sensitive to the compost treatments.

Table 6 presents the total fresh weight of 72 h old seedlings, in response to the seed treatment with 1% compost extracts. The fresh weight of corn was reduced when treated with almost all the compost extracts. Thus, indicating the highest reduction of seedling fresh weight (31.3% on average) after compost treatments when compared to the water control. Seedling fresh weight of red clover, soybean and Komatsuna were not affected by the majority of the compost treatments. However, these three species showed their highest sensitiveness to the inhibitory effects shown by compost No. 13 (hog-waste).

Table 3. Effect of 1% compost extracts on seed germination of forage crops (water control=100).

Comp. No.	CN* 24	CN* 48	CN* 72	SG 24	SG 48	SG 72	IR 24	IR 48	IR 72	AM 24	AM 48	AM 72
Cattle (CA)												
1	83	92	98	95	105	105	80	96	96	90	97	97
2	84	92	94	100	100	100	86	98	100	82	94	94
3	96	100	100	100	100	100	96	106	100	84	89	110
4	60	72	73	70	92	94	62	74	78	78	79	93
5	92	97	97	85	100	100	70	81	81	88	98	100
6	90	96	96	81	97	98	80	80	80	100	100	100
Chicken (CH)												
7	82	94	94	79	98	98	80	88	88	92	100	102
8	71	92	93	90	100	100	82	90	92	92	98	102
9	67	89	90	76	100	104	80	92	92	96	102	105
10	76	88	93	66	74	75	60	70	76	80	80	80
11	84	96	96	96	102	102	82	87	87	75	81	81
Hog (HG)												
12	92	94	94	72	75	75	90	94	94	94	98	98
13	81	90	94	90	100	100	78	91	91	88	100	100
14	80	97	97	74	85	85	58	82	85	90	100	100
Mean	81	92	94	84	95	96	77	87	89	87	97	98

Comp. No.	RC 24	RC 48	RC 72	AF 24	AF 48	AF 72	SB 24	SB 48	SB 72	KM 24	KM 48	KM 72
Cattle (CA)												
1	78	92	92	66	100	105	100	100	104	80	91	91
2	76	80	82	87	98	98	102	106	106	88	96	96
3	90	92	99	90	100	100	90	110	110	90	98	98
4	75	83	85	76	90	100	86	100	110	84	95	95
5	90	94	94	88	98	100	91	98	110	76	92	92
6	84	92	92	100	100	102	96	104	104	98	98	98
Chicken (CH)												
7	78	84	84	84	98	100	80	102	104	88	94	96
8	85	89	89	96	98	102	76	100	100	76	82	88
9	54	68	68	78	90	96	86	92	98	74	88	96
10	64	82	84	86	92	100	80	110	110	90	90	90
11	52	88	88	94	100	100	78	100	100	88	92	92
Hog (HG)												
12	82	88	88	92	94	110	96	104	110	70	88	88
13	68	74	76	70	76	88	98	98	100	86	88	94
14	80	84	84	90	102	102	100	100	100	88	90	90
Mean	75	84	88	86	96	100	90	102	106	85	92	95

Time after imbibition (h).

* see Table 1 for abbreviations.

The underlined data refer to the least germination % observed for each forage crop after compost treatments.

Table 4. Effect of 1% compost extracts on the shoot length of 72 h old forage crop seedlings (water control=100).

Compost No.	CN*	SG	IR	AM	RC	AF	SB	KM
Cattle (CA)								
1	116.1	93.1	95.6	98.3	115.0	55.5	81.8	111.4
2	112.8	93.0	102.7	88.3	109.8	50.6	91.8	109.1
3	98.0	105.5	115.6	104.2	119.6	80.2	101.0	118.7
4	77.5	82.9	86.4	108.6	94.8	57.3	104.6	114.3
5	114.8	111.5	90.3	99.6	110.6	110.7	115.7	106.8
6	94.0	96.6	98.5	99.2	110.0	110.8	110.4	100.9
Chicken (CH)								
7	71.2	104.7	101.0	96.2	94.0	100.8	104.6	106.7
8	102.9	79.5	89.9	75.6	77.3	88.0	115.4	113.0
9	62.6	62.4	56.4	69.7	80.3	62.0	88.1	60.6
10	59.6	54.3	52.0	59.4	61.6	96.0	67.7	50.6
11	74.3	90.0	73.6	88.8	72.0	115.6	118.3	109.3
Hog (HG)								
12	89.4	92.4	124.6	84.3	115.0	111.5	108.0	101.5
13	81.0	97.6	115.4	86.3	66.3	64.0	106.1	60.0
14	76.2	78.3	75.6	85.0	106.3	109.6	112.9	105.0
Mean	87.8	88.8	91.3	88.8	95.2	86.6	101.2	97.7
L. S. D.[a]	10.4	16.1	19.4	11.2	10.6	12.9	11.6	14.1

a) L. S. D. at p=0.05; * see Table 1 for abbreviations.
The underlined data refer to the least shoot length observed for each forage crop after compost treatments.

The linear correlation coefficents among the different parameters of the forage crops after compost treatment, are presented in Table 7. Seedling fresh weight was highly correlated with the seedling dry weight in all the test plants, except soybean. Shoot length of forage crop seedlings exhibited a significant correlation with at least one of the remaining parameters of the same crop species. In majority of the crops, seed germination was significantly correlated with one or more seedling growth parameters. The strength of the linear relationship shown between any two parameters of a particular crop differed from that of other crops.

The results obtained in response to the 4% compost extract treatments showed similar or much severe inhibitory effects on all above stated parameters (data not shown). The experiments indicated that the results on total seedling fresh weight are compatible with those on total dry weight, thus, the dry weight results are not discussed in this paper.

DISCUSSION

Four hour imbibition was found to be the best pretreatment for early and better germination of the test plant species. HADAS (1976) reported that seeds need a minimal hydration level in order to germinate. DUDECK and PEACOCK (1986) also stressed the importance of pregermination of Italian ryegrass and tall fescue seeds via imbibing. The different patterns of fresh weight gain exhibited by the different crop plant species (Fig. 1) may be at-

Table 5. Effect of 1% compost extracts on the root length of 72 h old forage crop seedlings (water control=100).

Compost No.	CN*	SG	IR	AM	RC	AF	SB	KM
Cattle (CA)								
1	100.1	80.8	80.6	97.5	50.0	85.6	72.5	101.2
2	91.5	90.2	70.2	70.0	65.6	80.6	92.8	105.0
3	93.1	100.5	89.4	92.9	75.4	95.2	84.3	108.9
4	84.5	76.6	65.8	86.1	84.0	99.4	86.7	90.9
5	89.6	113.1	65.7	78.1	99.3	96.7	93.0	103.4
6	119.4	108.5	66.9	83.6	97.8	97.5	105.3	100.6
Chicken (CH)								
7	86.3	112.7	83.4	92.1	75.0	100.0	78.8	80.2
8	120.5	56.5	60.1	109.6	88.4	85.2	105.9	83.0
9	62.8	100.4	66.1	84.0	83.9	59.4	104.9	88.3
10	69.8	60.4	56.6	62.3	47.1	66.0	68.1	88.8
11	94.0	115.7	82.4	71.1	71.0	85.9	102.6	106.3
Hog (HG)								
12	106.0	70.8	63.9	53.5	99.0	80.2	102.6	93.5
13	70.5	80.4	90.5	70.9	88.8	60.5	89.0	78.3
14	86.4	76.7	78.9	62.2	63.7	103.2	105.0	86.0
Mean	91.0	75.7	72.9	79.6	77.7	85.4	92.3	93.9
L. S. D.[a)]	11.2	18.7	12.5	19.3	12.9	11.5	10.1	12.4

a) L. S. D. at $p=0.05$; * see Table 1 for abbreviations.
The underlined data refer to the least root length observed for each forage crop after compost treatments.

tributed to the different seed characteristics such as seed size, metabolic activities, etc.

The different forage crop species exhibited different responses to animal-waste composts in terms of seed germination and seedling growth. The composts that retard the seed germination and seedling growth of a particular crop do not always affect the other plant species in the same manner. Soybean was the least sensitive to the inhibitory animal-waste composts (Tables 3, 4, 5 and 6). The results suggest of an interspecific difference among the forage crops in response to the animal-waste compost treatments. The difficulty of using a single indicator plant to detect compost toxicity in screening trials is suggested.

The seed germination and seedling growth of many forage crops were inhibited by some of the animal-waste compost treatments (Tables 3, 4, 5 and 6). The observations also explain that the response of the different parameters towards animal-waste composts are different, where seed germination, root and shoot growth results showed varied sensitivities to the compost treatments. These results explain of an inter-parametric difference in response of a particular crop to the animal-waste compost treatments. Thus, suggesting the difficulty of using a single parameter of a particular crop to detect the phytotoxicity of animal-waste composts.

The 1% concentration of compost extracts used in this study could be lower than the compost concentration in the soil solution in field. The compost concentration in the soil solution was estimated based on the assumptions, 10 t of composts containing 50% moisture was mixed with 1000 t of surface soil (1 ha, 10 cm depth, bulk density 1) with a moisture

Table 6. Effect of 1% compost extracts on the total fresh weight of 72 h old forage crop seedlings (water control=100).

Compost No.	CN*	SG	IR	AM	RC	AF	SB	KM
Cattle (CA)								
1	75.2	98.8	96.2	100.0	91.7	93.9	104.1	105.5
2	69.0	95.7	109.4	117.6	81.2	105.0	99.8	103.1
3	73.1	92.6	102.1	95.6	99.3	92.0	114.7	118.9
4	51.4	89.0	84.9	95.1	75.5	99.5	102.1	111.0
5	80.4	107.8	92.9	88.8	97.1	97.3	110.5	117.3
6	87.2	92.0	106.1	101.5	97.7	99.7	98.1	116.0
Chicken (CH)								
7	62.8	127.0	90.3	117.4	74.9	103.4	111.0	114.5
8	86.4	108.9	75.0	115.4	77.6	100.9	96.1	94.2
9	62.5	86.5	69.5	92.8	83.4	92.0	99.6	75.1
10	67.1	78.0	63.5	50.2	56.4	109.5	106.8	82.9
11	77.7	84.0	94.1	110.0	73.3	117.2	108.0	116.0
Hog (HG)								
12	69.0	119.5	87.4	108.1	72.9	116.5	97.5	111.0
13	80.0	100.0	70.2	83.0	73.0	69.1	92.5	73.4
14	79.5	67.7	68.6	66.7	65.0	108.7	108.3	114.2
Mean	68.7	94.6	86.4	95.9	79.9	100.3	103.5	103.8
L. S. D.[a)]	10.5	10.0	7.8	19.2	11.7	10.8	NS	16.1

a) L. S. D. at $p=0.05$; NS=Non significant

* see Table 1 for abbreviations.

The underlined data refer to the least fresh weight observed for each forage crop after compost treatments.

content of 12%. Thus, the concentration of composts in the soil solution in field was estimated as 4%. The results obtained after the treatment of 1% compost extracts suggest that the inhibitory effects may be more pronounced under field conditions with the application of large quantities of animal-waste composts.

Some informations that are documented on the effect of animal-wastes suggest that beef cattle manures affect the seed germination of barley seeds (STRAUCH, 1987), while raw and treated pig slurries were found to be affecting barley and wheat seed germination (RAMIREZ and GARRAWAY, 1982). This may be attributed to that the physiologically active material(s) that are found in composts affecting the germination process, which is still to be confirmed in this study.

The results explain that some composts dried at 60°C for 12 h still produce phytotoxic effects. This suggests the presence of fairly heat resistant material in animal-waste composts that are inhibitory to plant growth. The study was conducted under the conditions that recipient seeds are in direct and perfect contact with the compost extracts where water is readily available. It has been reported that the estimated germination under these conditions in the laboratory is in good accordance with the final germination determined under field conditions (HADAS, 1977). MCCORMAC and KEEFE (1990) also reported that preimbibiton of seeds in an aqueous medium before transfer to the germination conditions would serve to

Table 7. Linear correlation coefficients among the parameters.

	DW[a]	FW	RL	SL		DW	FW	RL	SL
CORN					RED CLOVER				
GE	.42	.69**	.26	.37		.55*	.58*	.18	.53*
SL	.45	.45	.61*	—		.54*	.60*	.17	—
RL	.54*	.52*	—	—		.22	.46	—	—
FW	.87**	—	—	—		.71**	—	—	—
SORGHUM					ALFALFA				
GE	.56*	.52*	.55*	.41		.51*	.72**	.44	.41
SL	.61*	.52*	.57*	—		.41	.55*	.40	—
RL	.52*	.13	—	—		.13	.32	—	—
FW	.71**	—	—	—		.77**	—	—	—
ITALIAN RYEGRASS					SOYBEAN				
GE	.38	.40	.54*	.52*		.16	.37	.52*	.20
SL	.48	.53*	.40	—		.15	.11	.67*	—
RL	.30	.31	—	—		.21	.38	—	—
FW	.81**	—	—	—		.47	—	—	—
AFRICAN MILLET					KOMATSUNA				
GE	.40	.27	.30	.34		.23	.16	.30	.19
SL	.62*	.40	.36	—		.67*	.84**	.52*	—
RL	.45	.43	—	—		.18	.54*	—	—
FW	.84**	—	—	—		.82**	—	—	—

a) GE: seed germination; SL: shoot length; RL: root length; FW: seedling fresh weight; DW: seedling dry weight

* significant at $p=0.05$ and ** significant at $p=0.01$ ($n=15$)

maximize imbibition stress and may therefore, more closely represent the situation in the field.

Table 7 explains that the relationship between any two parameters of a particular crop is different from other parameter combinations within the same crop spieces. In addition the results indicate that the correlation between any two parameters of a crop differs from that of other crops. These data also indicate of interspecific and inter-parametric differences of forage crops in response to the animal-waste composts, as explained in Tables 3, 4, 5 and 6.

The scope of this study was to identify the troublesome composts that are currently used as manures in Japan. Majority of the experiments conducted so far in Japan to detect phytotoxic animal-waste composts has employed Komatsuna as the indicator plant. However, this experiment has indicated the disadvantages of using a single plant in detecting compost toxicity. The various crop species used in this experiment may have different maximum acceptable levels of constituents in compost medium. Hence, the identification of toxic animal-waste composts with the use of a single indicator plant may not provide generalized conclusions. We suggest that it is important to test the plant species of interest, for their sensitiveness to any inhibitory effects of animal-waste composts prior to the practical usage.

This experiment has given evidence on toxic animal-waste composts that are used as manures in crop production in Japan. However, less attention has been paid on the toxicological aspects exhibited by these materials. The results suggest the need of further investigations on identification of the phytotoxic compounds in animal-waste composts and their mode of action that inhibit seed germination and seedling growth of forage crops. Research on these aspects will be of immense importance to develop the production technology of composts that minimize the phytotoxicity while maximizing the crop growth promoting properties of these animal-wastes.

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家畜排泄物コンポスト水抽出液で種子処理した飼料作物の生育反応における種間ならびに指標間差異

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家畜排泄物コンポストの作物生育阻害効果の検定方法について検討した。7種の飼料作物（トウモロコシ，ソルガム，イタリアンライグラス，シコクビエ，アカクローバー，アルファルファ，ダイズ）とコマツナの種子発芽と初期生育に及ぼす14種の家畜排泄物コンポストの阻害効果を室内実験で比較した。阻害効果の指標として，処理後72時間の発芽率，草丈，根長，新鮮重，乾物重を測定した。

コンポストの阻害効果は検定植物や指標間で異なり，単一の植物種の特定の測定指標でコンポストの阻害効果を判定することは困難であった。これらの結果は，コンポスト中の毒性成分とその作用が植物種間で異なりうることを示しており，阻害物質の同定とそれらの阻害作用機構を明らかにすることが必要である。