

Herbicidal Properties of Animal Waste Composts: Identification of Germination Inhibiting Substances

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ABSTRACT. *The selective germination-inhibitory properties of animal-waste composts were examined under laboratory conditions. These were identified using gas chromatography-mass spectrometry or thin-layer chromatography. Their biological activity was assayed using small-seeded legume and cereal plant species. Long-chain fatty acids, namely myristic, palmitic, stearic, oleic, linoleic and linolenic, were identified in the aqueous compost extracts. These acids were found at concentrations ranging from 5 to 15 mg l⁻¹ with linolenic as the dominant fatty acid. Phenolic acids, namely, p-coumaric, protocatechuic and m-hydroxybenzoic were found in minute quantities.*

The fatty acids inhibited the germination of alfalfa (Medicago sativa) and red clover (Trifolium pratense), but not that of Italian rye grass (Lolium multiflorum) and African millet (Eleusine corocana). Phenolic acids did not affect seed germination of the tested plants.

Long-chain fatty acids were the dominant group of germination-inhibitors in the tested animal-waste composts. These acids could selectively inhibit germination of the small-seeded legumes in a mixed stand with cereals.

INTRODUCTION

Animal-waste composts are widely used to maintain soil productivity in Japan and other Asian countries (Ando *et. al.*, 1990). Apart from their soil conditioning properties, some composts were also found to be phytotoxic (Strauch, 1987; Marambe and Ando, 1993).

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Cereal and leguminous forage crops are usually grown in Japan as mixed cultures. Unpublished results indicated that some animal-waste composts already in use in the Western Part of Japan, inhibit the germination rate of leguminous forage crops, such as, alfalfa and red clover, but not that of the cereal forage crops, such as, Italian rye grass and African millet. Seed germination is the most precarious stage of plant life. Thus, it was postulated that the application of these composts could result in a shift in plant populations in cereal-legume mixed stands.

Phenolic acids (Garraway and Ramirez, 1982), short-chain fatty acids (Cocucci *et. al.*, 1989) and osmotic cations (Wardle *et. al.*, 1992) have generally been considered as natural germination-inhibitors. However, Marambe (1993) reported that the osmotic cations could be excluded as a group of germination-inhibitors in composts. Therefore, the present study was conducted to identify the potent germination-inhibitors in animal-waste composts and to test their effects on germination of cereal and legume seeds.

MATERIALS AND METHODS

Crop seeds

Large and uniform seeds of two small-seeded cereal forages, namely, Italian rye grass [*Lolium multiflorum* (Lam) Turf.] and African millet (*Eleusine coracana* Gaertn.), and those of two small-seeded leguminous forages, namely, alfalfa (*Medicago sativa* Pers), and red clover (*Trifolium pratense* L) were selected from highly viable seed lots.

Composts

Three compost samples, one each from cattle, chicken, and hog-wastes were selected, based on their germination-inhibitory activities from the previous experiments (Marambe *et. al.*, 1991). These samples were selected from the Western part of Japan at the time of their dispatch to the market. The characteristics of the samples have been documented (Marambe *et. al.*, 1991).

The composts were extracted with deionized water at rates of 10, 40 and 80 g l^{-1} . The 40 g l^{-1} concentration was based on the calculations made on the normal application rate of composts in Japan (Marambe *et. al.*, 1991). The

extracts were centrifuged at 1600 g for 10 min. and filtered; and the filtrates were used as the test solutions. Deionized water was used as the control.

Germination tests

The seeds were imbibed separately in the respective treatment solutions for 4 h (Marambe *et. al.*, 1991), prior to transfer for germination. The rest of the procedure was conducted as described by Marambe and Ando (1993). Each petri dish contained 50 seeds, and the germination percentage was recorded after an incubation period of 5 days at 25°C.

Total water-soluble phenolic compounds in composts

The compost extracts were dried under reduced pressure in a rotary evaporator, and the residue was dissolved in 3 ml of ethanol. The qualitative estimation of phenolic acids were performed by thin-layer chromatography, (TLC) as described by Garraway and Ramirez (1982). The quantification of phenolic compounds was conducted colourimetrically, as proposed by Swain and Hillis (1959).

Water soluble fatty acids in composts

The compost samples were fractionated and analyzed, as described by Marambe (1993). The fractions were dried and methylated by treatment with BF₃. The qualitative analysis of fatty acids was carried out using a gas chromatograph-mass spectrometer (GCMS-QP1100EX, Shimadzu, Japan), and quantitative analysis was conducted using a Shimadzu GC 14A gas chromatograph.

Germination assay of the identified substances

The solutions of authentic samples of the identified substances were made conforming to the concentration of the fatty acids in compost extracts (0 to 15 mg l⁻¹ in 0.5% methanol). The control treatment also had the same volume of methanol. The pH of all the test solutions was adjusted to 7 (Marambe *et. al.*, 1991). The solutions were then assayed for their germination- inhibitory activity as discussed earlier.

The germination tests were conducted in a complete randomized design with ten replicates. The results were subjected to statistical analysis after arc-sine transformation.

RESULTS AND DISCUSSION

Effects of animal-waste composts on seed germination

The germination percentage of red clover and alfalfa was reduced by values greater than 25 and 30%, respectively, in the treatment with 10 gl^{-1} extracts of all the composts when compared to the respective controls (Figure 1). This indicates that the composts are inhibitory to germination of the tested leguminous plant species at the normal rates of field application (Marambe *et al.*, 1991). The inhibitory effects were enhanced with the increase in concentration of extracts. The germination percentage of red clover was reduced by 75% when treated with 80 gl^{-1} extract of hog-waste compost. However, the seed germination percentage of the cereal plant species was not affected by the forms of treatment. The interspecific differences observed in this study confirmed the earlier reports by Marambe *et al.*, (1991) which suggest that the animal-waste composts selectively inhibited the germination rate of small-seeded legumes.

Effect of phenolic acids in composts on seed germination

Very low quantities of total phenolic compounds were found in the tested composts (less than 2 mg l^{-1}). Three phenolic acids, namely, p-coumaric, protocatechuic, and m-hydroxybenzoic were identified by TLC experiments by comparing the r_f values of the authentic samples (Marambe and Ando, 1992).

The phenolic acids are generally considered as a major group of germination-inhibitors in animal-excrements (Garraway and Ramirez, 1982). The solutions of the identified phenolic acids did not show any significant germination-inhibitory effects even at 10 mg l^{-1} . However, investigations on the synergistic effects of the phenolic acids were not carried out as explained by Blum *et al.*, (1985). The results of the present experiment are in agreement with Marambe and Ando (1992), who reported that phenolic acids are germination-inhibitory when present at concentrations as high as 100 mg/l . Thus, these phenolic acids may not be a dominant group of

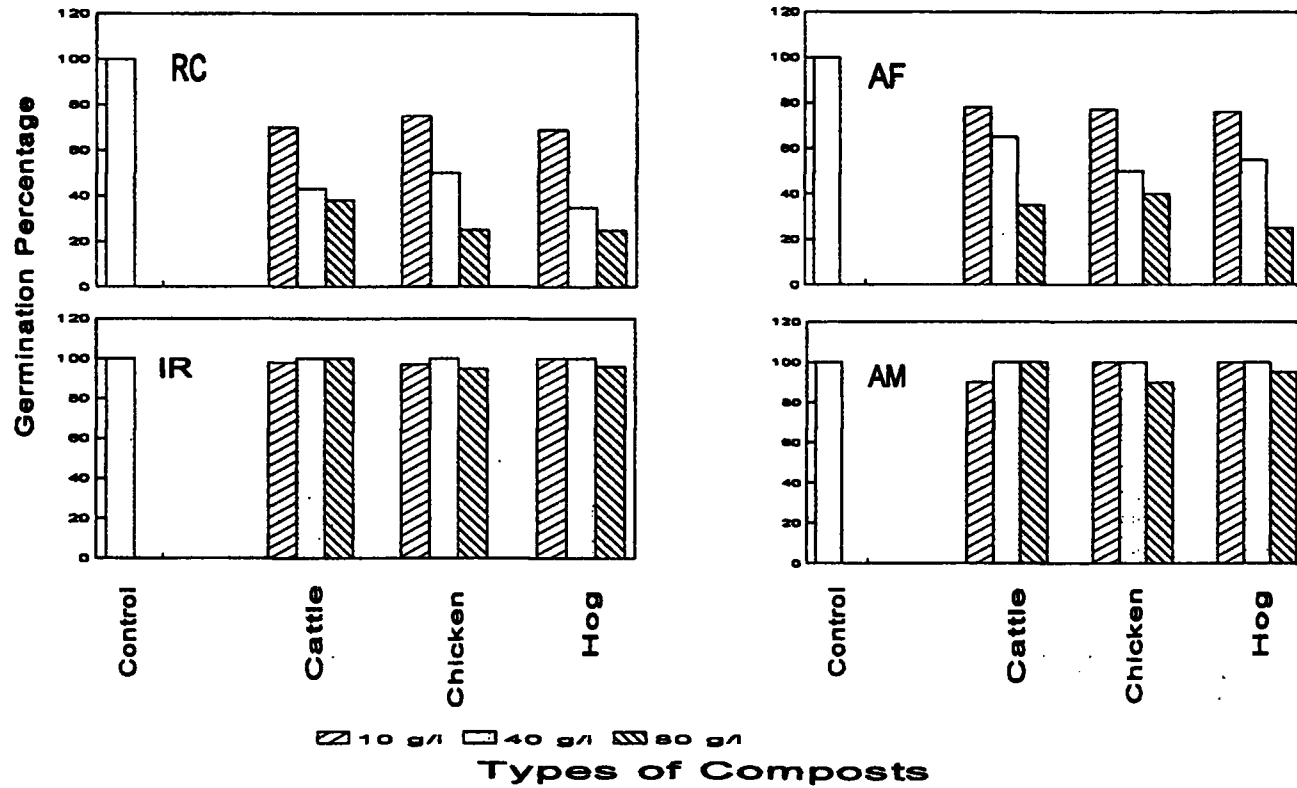


Figure 1. Effect of different concentration of animal-waste compost extracts on the germination rate of plant species. RC - Red Clover; AF - Alfalfa; IR - Italian rye grass; AM - African Millet

germination-inhibitors in the composts.

Effect of fatty acids in the composts on seed germination

The highest concentration of long-chain fatty acids was found in the extract of hog-waste compost followed by chicken- and cattle-waste extracts (Table 1). The types of long-chain fatty acids varied with the type of compost. Linolenic acid was the dominant fatty acid in the tested samples. Mass spectrum analysis indicated that long-chain fatty acids with chain lengths ranging from 12 to 22 carbon atoms were present in the composts. Short- or middle-chain fatty acids were not observed in the mass spectrum analysis.

Table 1. Quantitative analysis of the water soluble long-chain fatty acids in composts.

Fatty acid	Composts		
	Cattle	Chicken (ug/g dry compost)	Hog
Myristic	ND*	7.2	17.4
Palmitic	10.0	32.0	55.8
Stearic	ND	14.8	38.6
Oleic	ND	14.0	4.6
Linoleic	ND	7.2	16.4
Linolenic	95.4	119.4	107.0
Total	105.4	194.6	259.8

*ND - Not detected.

The authentic samples of the long-chain fatty acids significantly reduced the percentage of seed germination of alfalfa, and red clover at 5 mg^l⁻¹ concentration (Figure 2). The 10 mg^l⁻¹ concentration of the fatty acids reduced the germination of legumes by 50%. Only 1-2% of these legume seeds germinated when treated with 15 mg^l⁻¹ solutions of the fatty acids. The results support the findings of AlSadaawi *et. al.*, (1983) who also reported the germination-inhibitory effects of long-chain fatty acids extracted

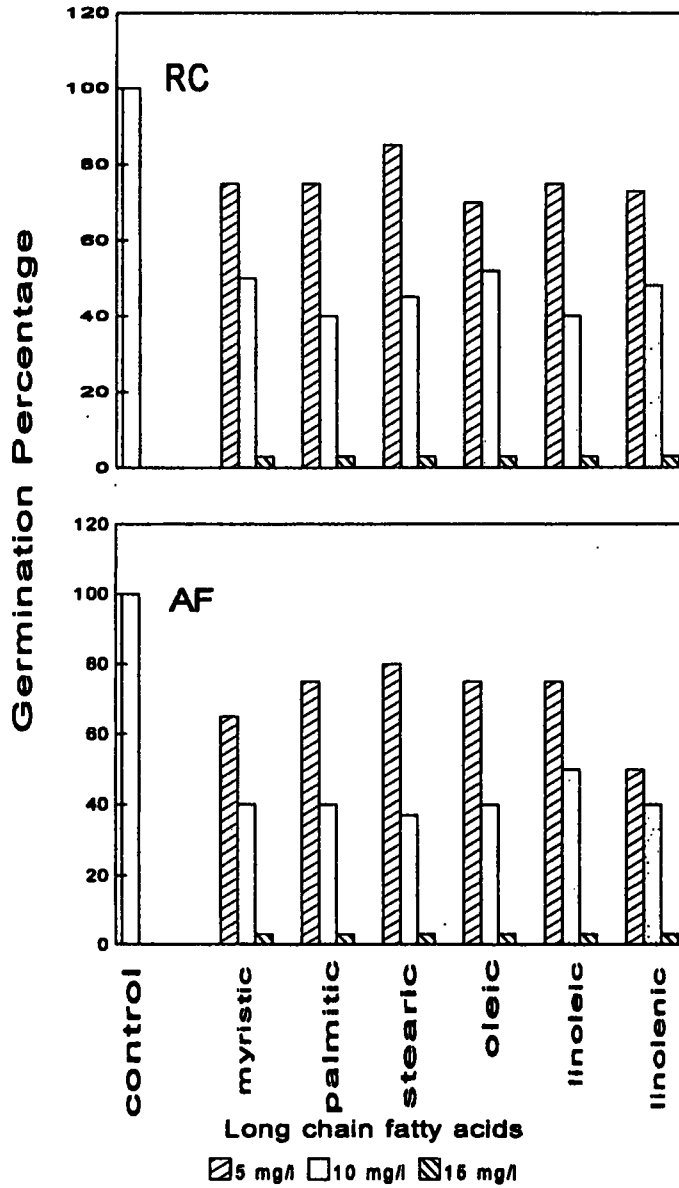


Figure 2. Effect of different concentrations of long-chain fatty acids on the germination rate of plant species. RC = Red Clover; AF = Alfalfa (The vertical bars indicate LSD at $p = 0.05$)

from plant sources. The seed germination rate of cereal plant species was not affected by the fatty acids at these concentrations.

The results indicate that fatty acids selectively inhibited the germination percentage of legume seeds. However, all the long-chain fatty acids in composts were not identified, because of the lack of authentic samples. Marambe (1993) reported that inhibition of the synthesis of alpha-amylase may be a possible mode of action of these germination-inhibiting substances.

CONCLUSIONS

The results of the present experiment suggest that long-chain fatty acids are a potent group of germination-inhibitors in the animal-waste composts. These substances could control population growth of small-seeded legumes in a mixed stand with cereals, and thus, could be used successfully in management of lawns and turfs. However, investigations on the effects of these substances under field situations are needed, prior to generalization of the conclusions. The potential for the use of these composts/long-chain fatty acids as selective herbicides is indicated by the experimental results.

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