

example, *T. harzianum* isolate no. 1 clusters with *T. viride* isolate no. 1, while *T. harzianum* isolate no. 2 clusters with *T. viride* isolate no. 2. Similarly, *T. virens* isolate no. 1 is more close to *T. koningii* isolate no. 1, than to *T. virens* isolate no. 2, while *T. pseudokoningii* isolate no. 2 is more close to *T. hamatum* isolates, than to *T. pseudokoningii* isolate no. 1. Since RAPD is often considered to be less reliable than RFLP data, we also analysed the RFLP in the amplified ITS1–5.8S–ITS2 region. An annealing temperature of 59°C was found to be suitable for the amplification of ITS1–5.8S–ITS2 region from all the isolates with good product yield and minimum non-specific amplifications. The product size was approximately 600 bp, and there was size variation across the isolates (Figure 2). At all the three annealing temperatures, we could see two bands only with the *T. viride* isolate no. 1. Digestion of this product with five tetra-base cutters (*Mbo*I, *Hae*III, *Taq*I, *Sau*3AI, *Msp*I) revealed polymorphism in the ITS1–5.8S–ITS2 region (Figure 2). All the isolates could be divided into broadly four groups (Table 2), which again showed the overlapping in species identification of these strains, e.g. *T. pseudokoningii* isolate no. 2, and *T. koningii* isolate no. 2 grouped with *T. hamatum* isolates.

The present analysis questions the identity of *Trichoderma* isolates maintained in two of the Indian type culture

collections. This is not surprising given the fact that these were identified using morphological data, which, as a taxonomic tool for *Trichoderma* spp., have been confusing. It is therefore proposed that all the isolates of *Trichoderma* spp. deposited in Indian type culture collections be re-identified using the currently available molecular tools (e.g. sequencing of the part of rDNA), in order to effectively utilize these fungi of immense agricultural, biotechnological and industrial importance.

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Phenolics in elephant dung: a complex zoochoric system

While anemochory has brought in its wake a wide assortment of engineering perfection in wind-borne seeds, zoochory involves many complex aspects of chemistry, for example, the chemistry of fruit pulp^{1–3}. Even seed dispersal through elephant dung^{4–6} is intricately associated with the chemical properties of the dung. For example, herbivore dung is known to contain phenolics and these compounds influence growth.

Apart from dispersal per se, the effect of dung as manure on the germinated

seedlings has been considered⁴. But elephant dung is a very complex system, with so many possible chemical agents which might act as stimulators or inhibitors of germination and/or seed growth. These may be the metabolic products of the elephant's own physiological system and chemicals from the very large amount of undigested/partially digested vegetal remains, a characteristic feature of elephant and rhino dung. Such dung are expected to contain phenolics. Since a large quantity and variety of phenolic

substances occur in the plant world, we have investigated the possibility of phenolics in elephant dung exerting an influence on seedling growth. This would be apart from any nutritive effect that the dung as a source of manure might exert.

Elephant dung was collected from Chandak Elephant Reserve, Orissa and the aqueous extract was filtered and tested on IET rice grains showing 100% germination. A standard volume of water (10 ml) was put in petri dishes containing Whatman filter paper on which the

Table 1. Effect of elephant dung extract on germination and seedling growth of rice. Mean of 40 seedlings recorded in mm

Test extract	Shoot length				Root length				Per cent stimulation/inhibition	
	M	SD	SE	t value	M	SD	SE	t value	in SL	in RL
S.1	29.5	4.6	0.73	-12.0	46.0	6.6	1.05	12.4	22.9	12.7
S.1 (1/3)	23.0	7.3	1.16	2.5	41.7	11.1	1.7	12.8	-3.7	-13.5
S.1 (3/2)	12.0	5.1	0.88	19.2	26.1	9.3	1.6	37.7	-31.0	-30.2
2.S.2	12.9	3.1	0.58	18.2	27.3	3.8	0.7	6.2	-11.0	-18.7
S.2	26.4	5.5	0.85	-2.1	44.6	6.05	0.93	-27.3	6.8	15.8
S.2/2	28.7	5.6	0.87	-19.2	47.3	9.2	1.42	-12.5	16.1	22.8
S.2/4	27.4	5.3	0.82	-17.5	49.3	4.7	0.72	-45.2	10.9	28.0

+, Stimulation; -, Inhibition.

The observed difference in shoot and root length between control and test extract is statistically significant at 0.01 level (two-tailed).

M, SD, SE, SL and RL indicate mean, standard deviation, standard error, shoot length and root length respectively.

S₁ = 180 g/350 ml; S₂ = 241 g/350 ml.

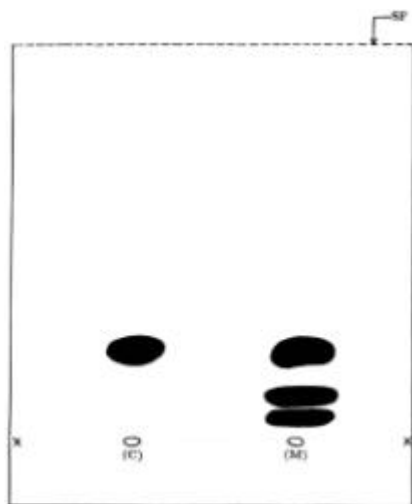


Figure 1. TLC run of concentrated elephant dung extract (M) along with standard caffeic acid (C). The uppermost spot of M is fluorescent like that of caffeic acid. Solvent system: chloroform : acetone :: 90 : 10.

control (rice grains) were placed. Equal volume of aqueous dung extract was put on the experimental seeds.

The stock solution of elephant dung extract (S) was further diluted to different degrees. S was an extract of 180 g in 350 ml. In this first experiment, two control sets were set up in order to study more accurately the individual variations in control seedlings. Mean difference in shoot length is 2.5%, and in root length it is 0.8%. Compared with the controls most of the treatment data seem to be acceptable as stimulation or inhibition (Table 1). The results show that depending on the concentration, the same dung extract can stimulate or inhibit.

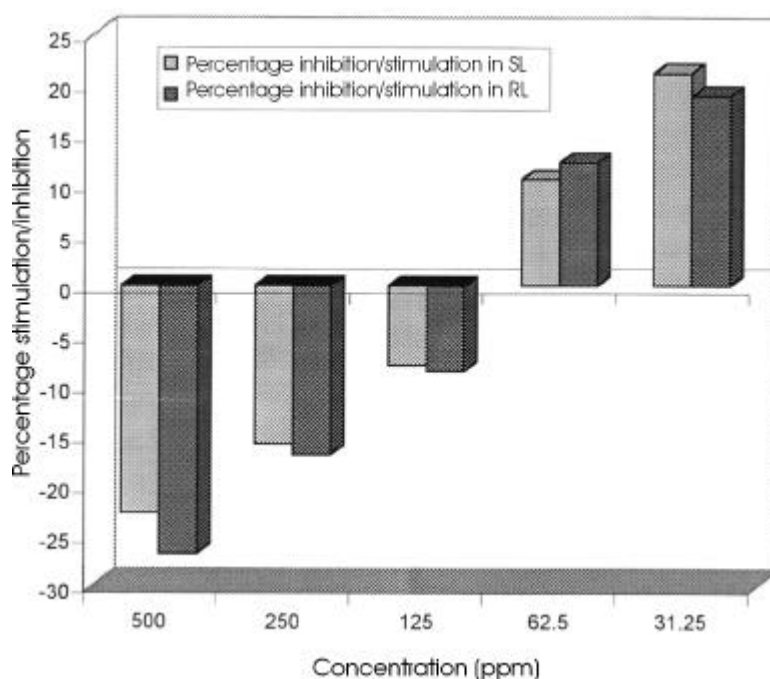


Figure 2. Effect of standard caffeic acid at different concentrations on germination and seedling growth of rice. +, Stimulation; -, Inhibition.

The putative phenolics were then tested with paper chromatography following an earlier work on the pulp phenolics². Three spots stained by AgNO₃ were visible, one of which coincides with standard caffeic acid (Figure 1). Standard caffeic acid shows concentration-dependent inhibitory and stimulatory activity (Figure 2). Ten mg of caffeic acid was dissolved in 20 ml of distilled water (= 500 ppm). Further dilutions of 250, 125, 62.5 and 31.25 ppm were made. At 500 ppm concentration, it

shows 22.7% inhibition in shoot length and 26.9% inhibition in root length. At 250 ppm it shows 15.8% and 16.9% and at 125 ppm 7.9% and 8.5% inhibition in shoot length and root length respectively. Then it exhibits stimulatory result. At 62.5 ppm, it shows 10.6% and 12.3% stimulation in shoot and root length respectively. At 31.25 ppm, it shows 21.2% and 18.7% stimulation in shoot and root length respectively.

This effect further supports the assumption that phenolic contents in the ele-

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phant dung can indeed either stimulate or inhibit seedling growth. This would depend on the species and the quantity of vegetal remains and the volume of moisture (from rainfall and dewfall) in the dung. Thus, apart from physical dispersal, the elephant dung constitutes a complex chemical system which interacts with seedling growth.

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