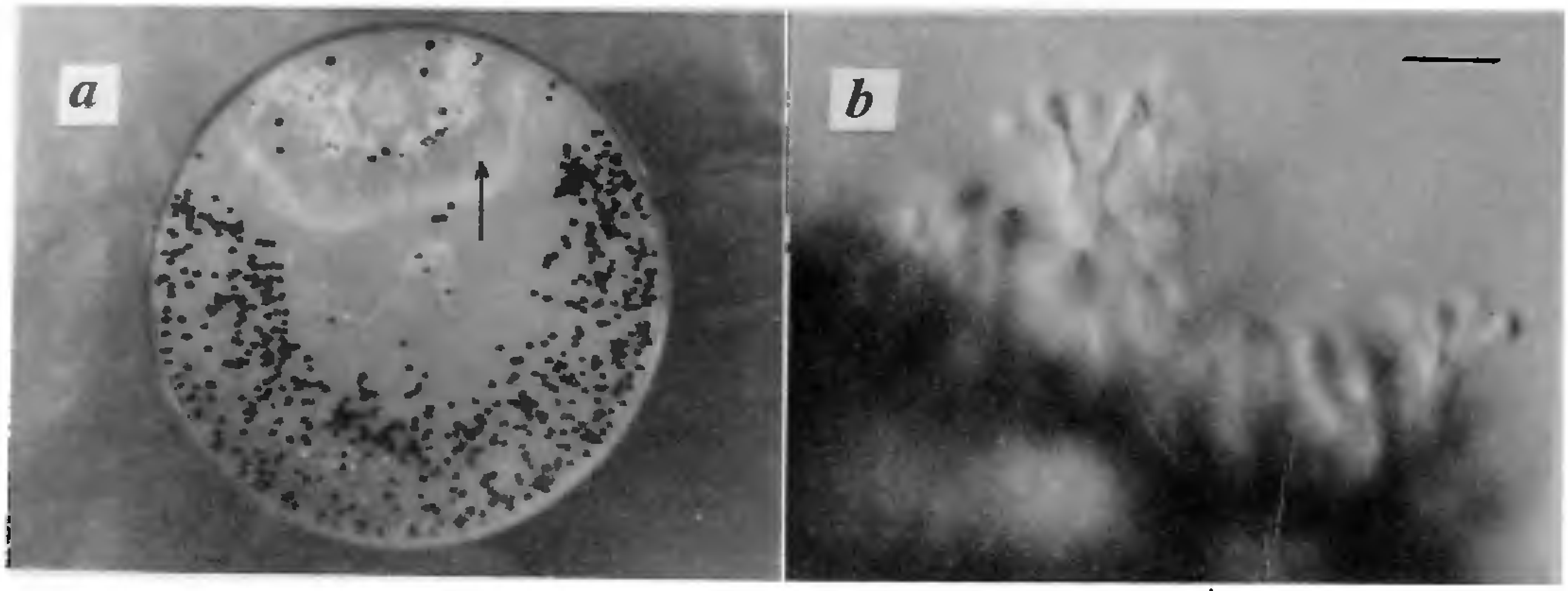

Figure 1. *a*, Abundant hymenium formation around *Bacillus subtilis* colony. *b*, Hymenium with basidia, each bearing four basidiospores. (Bar = 10 μm)

This is in addition to the role of *Cyperus rotundus* reported earlier[2]. (ii) The metabolites of *B. subtilis*, although effective against vegetative growth of *S. rolfsii*, do not have any effect on its sexual stage. (iii) More importantly, this observation questions the wisdom of the use of such biocontrol agents for plant disease control as it may lead to the creation of a wide genetic variability in pathogens with unpredictable results as seen in the case of *Phytophthora infestans* where oospores were induced by *Trichoderma viride*, a potential biocontrol agent[3]. The two types of hyphae, one responsible for sexual reproduction and the other for vegetative growth in *S. rolfsii* following treatment with *B. subtilis*, are being reported for the first time. *B. subtilis* may help in elucidating reasons(s) for cellular differentiation in *S. rolfsii*. This is the first report of such an interaction between a fungus and a bacterium.

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Chemical ecology of certain tropical fruit pulp

It is well known that the fruit pulps of many tree species contain inhibitors/stimulators of germination[1]. This makes sense in the ecological perspective, for, such inhibitors would not only prevent premature germination of seeds in the fruit still on the tree but also of those dropped from the tree and lying below. Delayed germination should be in the interest of the tree because zoochoric agents (birds, mammals, etc.) can remove the inhibitors through their alimentary canal while dispersing seeds to distant points[2]. It is therefore expected that natural selection should favour metabolism of germination inhibitory substances and of nutrients for zoochoric agents. It is thus of interest to study the germination-inhibitors of relatively little known tropical species. Our investigations have revealed not only the inhibitors but also, unexpectedly, stimulators in the pulp. These substances seem to occur even when, theoretically, their presence is not necessary.

Bird dispersal of *Ficus bengalensis* seeds has been reported[3]. After establishing that germination of *F. bengalensis* seeds is absolutely inhibited in presence of the pulp, aqueous extract of the latter was also found to be strongly inhibitory (Brahmachary and Midya, unpublished). Preliminary experiments suggested that such an extract also strongly inhibits monocots (standard rice grains).

To understand the inhibitory action, 10 g of pulp was macerated in 10 ml water, centrifuged, filtered and recentrifuged and the supernatant solution (stock 1) was taken. 40 standard rice grains (with practically cent per cent germination) were used in each assay set. Stock 1 exerted 100% inhibitory effect while dilutions (2, 4, 8 and 16 times) caused lesser and lesser inhibition.

Through paper chromatography it was found that the aqueous extract contained inhibitory substances in the form of phenolics as well as unknown stimulators (Brahmachary, unpublished).

One of the ecological scaffoldings in Namibia is the Kameeldoring or Camel thorn, *Acacia erioloba*. The large heavy pods drop under the tree and seed dispersal is possible only through zoochoric agents: wild animals such as the elephant, kudu (antelope) and

Table 1. Effect of *Acacia erioloba* pulp extract on germination and seedling growth of rice

No. of observation	Shoot length			Root length			Percentage inhibition	
	M	SD	SE	M	SD	SE	in SL	in RL
1	7.4	1.71	0.31	12.1	3.73	0.68	48.0	69.2
2	7.4	1.86	0.34	9.0	3.88	0.70	54.1	75.8
3	7.2	1.33	0.26	11.7	3.29	0.68	46.2	70.0

M, mean; SD, standard deviation; SE, standard error; SL, shoot length; RL, root length.

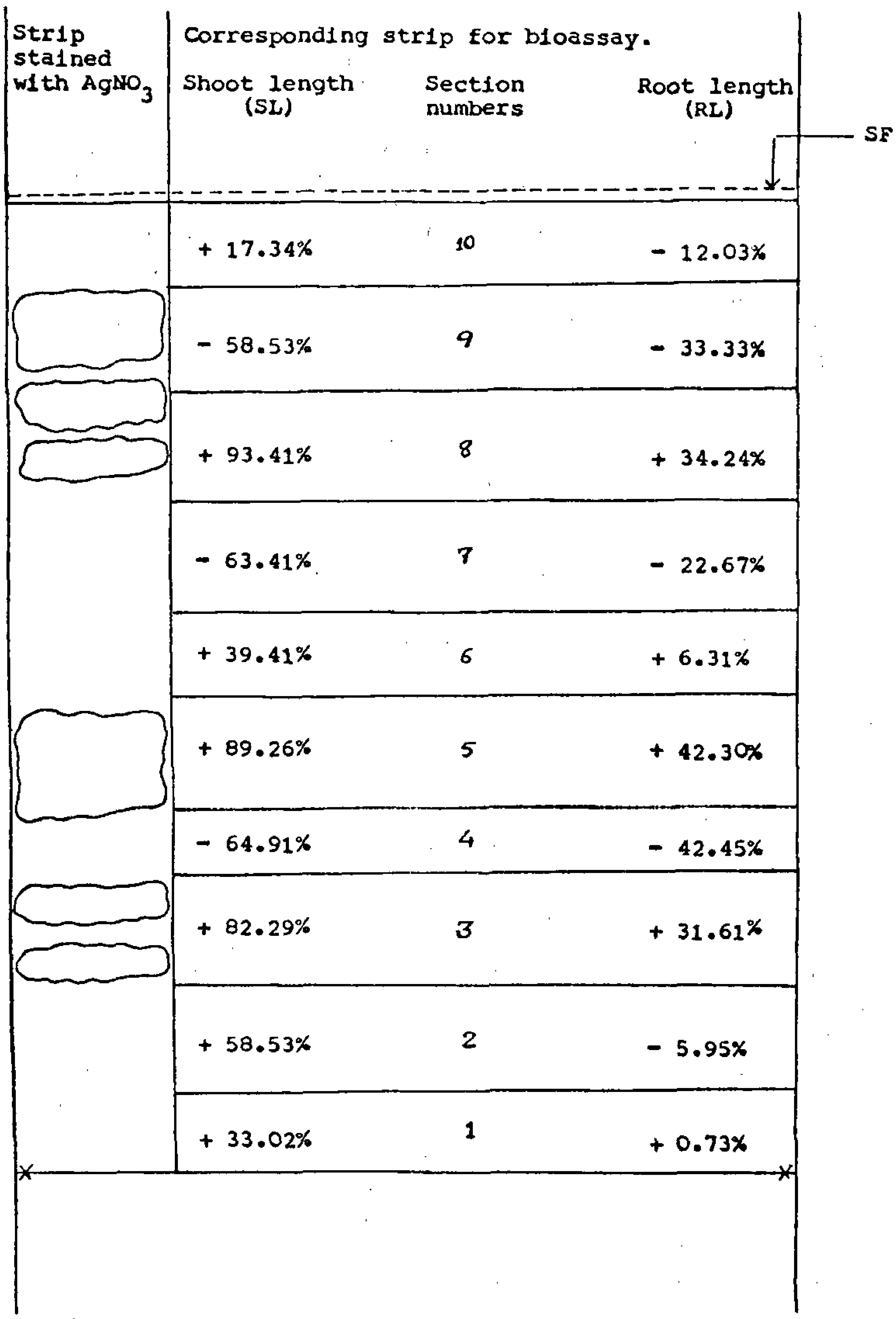

Figure 1. Inhibitory and stimulatory influences of the different sections of the chromatogram developed with 0.5 ml of *Acacia erioloba* pulp exptract. Solvet – butanol:acetic acid:water::4:1:2.2, widths of sections 2, 3, 5, 7, 8, 9 is 2 cm; 1, 6, 10 is 1.5 cm and 4 is 1.1 cm (Mean of 25 seeds recorded in mm.)

domestic animals like cattle and horse. The pods dropped in the Namibian summer have to perforce lie dormant throughout the dry winter till the rains arrive. Apparently during this long period, zoochory is likely to be executed and for lack of water no premature germination is possible at all. It is therefore unnecessary for the tree to metabolize inhibitors in the pulp.

For the preparation of pulp extract, 5 g of pulp of *A. erioloba* was macerated in 60 ml water and centrifuged and 35 ml supernatant used. Isolation and detection of the putative active compounds in the fruit pulp extract were attempted with the help of paper chromatography (Whatman no. 3). For phenolic compounds, the solvent was *n*-butanol:acetic acid:water::4:1:2.2. Silver nitrate reagent was used for staining phenolics[4].

For bioassay, 50 rice seeds (var. IET 1444), after sterilization in 0.1% mercuric chloride were used for each set. Control was maintained by treating with equal volume (10 ml) of distilled water. After 3 days, shoot and root length in the control and treated set were measured.

Bioassay was attempted after preparative chromatography (using solvent system *n*-butanol:acetic acid:water:: 4:1:2.2) with 0.5 ml pulp extract. One vertical section of the chromatogram was stained with $AgNO_3$ reagent[4], the remaining larger unstained portion was divided into 10 unequal sections depending on the location of spots. Height of the chromatogram was 17.7 cm. Sections 2, 3, 5, 7, 8, 9 were 2 cm; sections 1, 6, 10 were 1.5 cm and section 4 was 1.1 cm. 25 rice seeds were placed on each section and the corresponding control (same sized pieces cut out of a paper after blank run in identical conditions). Three such experiments were performed. Shoot and root length were measured after 3 days. Six spots were visible after staining with $AgNO_3$ reagent.

Two sets of measurements were performed, the treated set with aqueous pulp extract of *A. erioloba* and the control with distilled water. Shoot and root length in the two sets were measured after 3 days. Table 1 shows inhibition in shoot and root length in a treated set.

Bioassay on the different sections of preparative chromatogram shows inhition of both shoot and root length at sections 4, 7 and 9 (Figure 1). Stimulation of shoot length was observed in sections 1–3, 5, 6 and 8. The effect on root length is less but still not negligible (sections 3, 5, 8). Three phenolic spots were observed in sections 8, 9, one in section 5 and two in section 3.

Inhibition in shoot and root length caused by *A. erioloba* pulp extract is not 100% at the dilution used (higher conc. is not feasible because of the nature of the pulp) but is nevertheless considerable. It is approx. 46–54% (shoot length) and 69–75% (root length).

Three inhibitors and three stimulators are present in the pulp extract of *A. erioloba*. Incidentally, all three stimulators are clear because they are separated by inhibitors.

Six phenolic spots have been found, of which two (in sections 5 and 9) coincide with a stimulator and an inhibitor respectively. Nevertheless, the possibility remains that the stimulatory and inhibitory agents are unknown substances coinciding with these phenolics. Further characterization of these phenolics might shed more light on this problem. The stimulator ranging from 1 to 3 is maximal at section 3, it is also considerable at section 2 but there is no phenolic spot trailing up to section 2. It is, therefore, more likely that this stimulator is not either of the two phenolics revealed in section 3.

Inhibitors in the fruit pulp prevent premature germination and as such may have been selected for, because it is to the interest of both the mother plant and seeds. Parent–offspring conflict in plants is an important subject that has drawn considerable attention[5] and it is even possible that pulp inhibitor or seed dormancy reduces such conflict.

Again, mangrove fruit pulp should contain no inhibitors because the seed germinates in the fruit while attached to the tree; it is even likely that there may be stimulators. But it has been found that here, too, there are inhibitors, though, apparently, the mangrove seedling can overcome the effect of these[6] and slight stimulators of rice grains.

The above results show that inhibitors are present even in those fruit pulp where there is no such theoretical necessity. Furthermore, there are more than one inhibitor and stimulator, the synergistic effect of which is inhibition.

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Why do plants possess laxatives?

The functional significance of fruit pulp in plants could extend beyond merely offering nutrient rewards for their dispersers. Murray *et al.*[1] showed that fruit pulp of *Witheringa solanacea* increases the passage of seeds through the gut of the avian disperser *Myadestes melanops*. They found that seeds encapsulated in the agar blocks treated with the pulp extract passed significantly faster through the gut of its disperser than those encapsulated in untreated agar. The seeds that passed rapidly through the gut germinated better and remained viable for a longer time than those that passed slowly. Murray *et al.*[1] proposed that reducing the seed retention time might increase the fitness of the plant and hence that selection might favour the evolution of laxative chemicals in the fruits so as to enhance seed passage rates. Thus seed retention time appears to be an important component of fitness in plants. Accordingly we predict that selection shall favour the evolution of laxative chemicals predominantly in fruits and seeds (in structures such as seed coat and aril) of the species that are especially dispersed by animals as opposed to those dispersed by wind, water or passive means; unlike animal-dispersed species, wind and passively dispersed species do not require such laxatives. Here we test this prediction by analysing the association between the occurrence of laxative property in plants and their respective dispersal modes.

Several traditional health care systems, such as Ayurveda and Siddha, predominantly use plant products for curing human ailments. These systems of medicine have been very popular in the Indian sub-continent and even today enjoy a substantial patronage. We surveyed three compendia of these health systems[2–4] for information on plant species and their parts exhibiting laxative property. Plants listed to serve as purgatives were considered to possess laxative property and the dispersal modes of these species (animal or wind or passive) were obtained from the *Flora of the Presidency of Madras*[5] and from our own data source[6]. We then tested for the independence of the laxative property of the species with their dispersal mode. For this we computed the expected frequency of the species with different dispersal modes based on the data provided by Lokesha *et al.*[6] on the dispersal modes of a set of 770 species of angiosperms. These frequencies were compared with those with the laxative properties.

Nearly 60% of the 114 species with laxative property in their seeds and fruits are dispersed by animals (Table 1). Significantly more animal-dispersed species exhibited laxative