

TABLE I

Effect of incubation in Paraseodymium chloride on the stomatal regulation in isolated peelings of *A. tenuifolius*

Concentrations in M	Incubation for 3 hours		Incubation for 24 hours	
	Pore width in $\mu$	Starch test	Pore width in $\mu$	Starch test
0	3.4 $\pm$ 0.6	+	3.4 $\pm$ 0.6	+
0.1	0.0 $\pm$ 0.0	+	0.0 $\pm$ 0.0	+
0.05	2.7 $\pm$ 0.6	Slight	0.0 $\pm$ 0.0	+
10 <sup>-2</sup>	3.75 $\pm$ 0.7	—	6.9 $\pm$ 0.7	—
10 <sup>-3</sup>	3.3 $\pm$ 0.6	Slight	5.4 $\pm$ 0.7	—

TABLE II

Effect of spray treatment with Paraseodymium chloride on the stomatal regulation in *A. tenuifolius*

Concentrations in M	After 24 hours		After 72 hours		After 120 hours	
	Pore width in $\mu$	Starch test	Pore width in $\mu$	Starch test	Pore width in $\mu$	Starch test
0	3.4 $\pm$ 0.6	+	3.4 $\pm$ 0.6	+	3.4 $\pm$ 0.6	+
0.01	3.4 $\pm$ 0.6	+	8.1 $\pm$ 1.3	—	12.3 $\pm$ 2.0	—
0.5	0.0 $\pm$ 0.0	+	0.0 $\pm$ 0.0	+	2.5 $\pm$ 0.6	+

The general technique employed in the present study has been fully described in a previous paper<sup>7</sup>. Besides isolated epidermal peelings the experiments were also performed with plants growing in the field. The isolated peelings were incubated in different concentrations of Paraseodymium chloride (0.05, 0.01, 10<sup>-3</sup> and 10<sup>-4</sup> M) and in distilled water for control. The observations were taken after 3 and 6 hours of incubation.

For intact plants, the solutions were sprayed only once and the observations were taken after 24, 72 and 120 hours of spray treatment. Only two concentrations (0.5 and 0.01 M) were used in these experiments, with 0.01% Triton as surfactant.

The effect of Paraseodymium chloride on stomatal regulation in isolated peelings has been shown in Table I. It is evident from Table I that the maximum opening (7.0  $\mu$ ) was found in peelings incubated in 10<sup>-2</sup> M solution, when observed after 24 hours. In higher concentrations plasmolysis in epidermal cells took place resulting in the closure of stomata. A negative test for starch was observed in open stomata.

In intact plants the maximum opening (12.3  $\mu$ ) was found after 120 hours of spray treatment, after which it started reducing (Table II). A positive test for starch was observed in open stomata. In higher concentrations the stomata closed down due to plasmolysis (Table II).

It is evident from the foregoing observations that rare earth elements have also some role on the regulation of stomatal movement in *A. tenuifolius*. Some workers have already reported that only monovalent ions are responsible for opening of stomata<sup>6</sup>. Bivalent and trivalent ions have also been shown to effect stomatal opening<sup>2</sup>. All these metallic cations invariably cause the hydrolysis of guard cells starch, which helps in the opening of stomata<sup>1,2,4,6,8</sup> and similar results were also observed with rare earth elements in the present study. The effect of Paraseodymium chloride is slower on intact plants. Thus, it appears that not only monovalent, divalent and trivalents but also rare earth elements can cause the opening of stomata.

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#### A METHOD FOR THE DETERMINATION OF KERATINOPHILIC MOLDS

KERATINOPHILIC fungi in general are potential danger to man and animals. These fungi can be isolated from the soil with the help of baits consisting of keratinized substrates like hair, feathers, hoofs, nails, etc. Griffin<sup>2,3</sup> noted the initial colonization of sterile hair by a succession of molds which often did not show keratinolytic properties. These were the fast growing fungi whose spores were

abundant in the soil. On the other hand, the fungi which possess the ability to utilize the keratin of the hair as sole source of nutrient or capable of attacking and digesting hair keratin can be confirmed by the method described here.

The organisms isolated from the colonized hair were cultured on the Sabouraud dextrose agar medium. To evaluate the keratinolytic property, the individual isolate was grown on a sterile hair tied on a glass rod bent to a particular shape A as shown in Fig. 1. Hair was inoculated in the middle of its length simply by transferring the fungal spores or mycelial bits with the help of a sterile needle. The glass rod was suspended with the help of a cork in a large specimen tube having sterile moist cotton D at the bottom. To reduce the loss of water a small quantity of glycerine was mixed with it. The whole assembly was sterilized prior to inoculation with the fungus. After 20 to 25 days of incubation at 26° C (temperature and incubation period can vary according to the test organism) the test fungus developed a colony (Fig. 1 C) on the hair and if growth is continued the hair would break, indicating the digestion of the keratin of the hair and its disintegration by the test fungus.

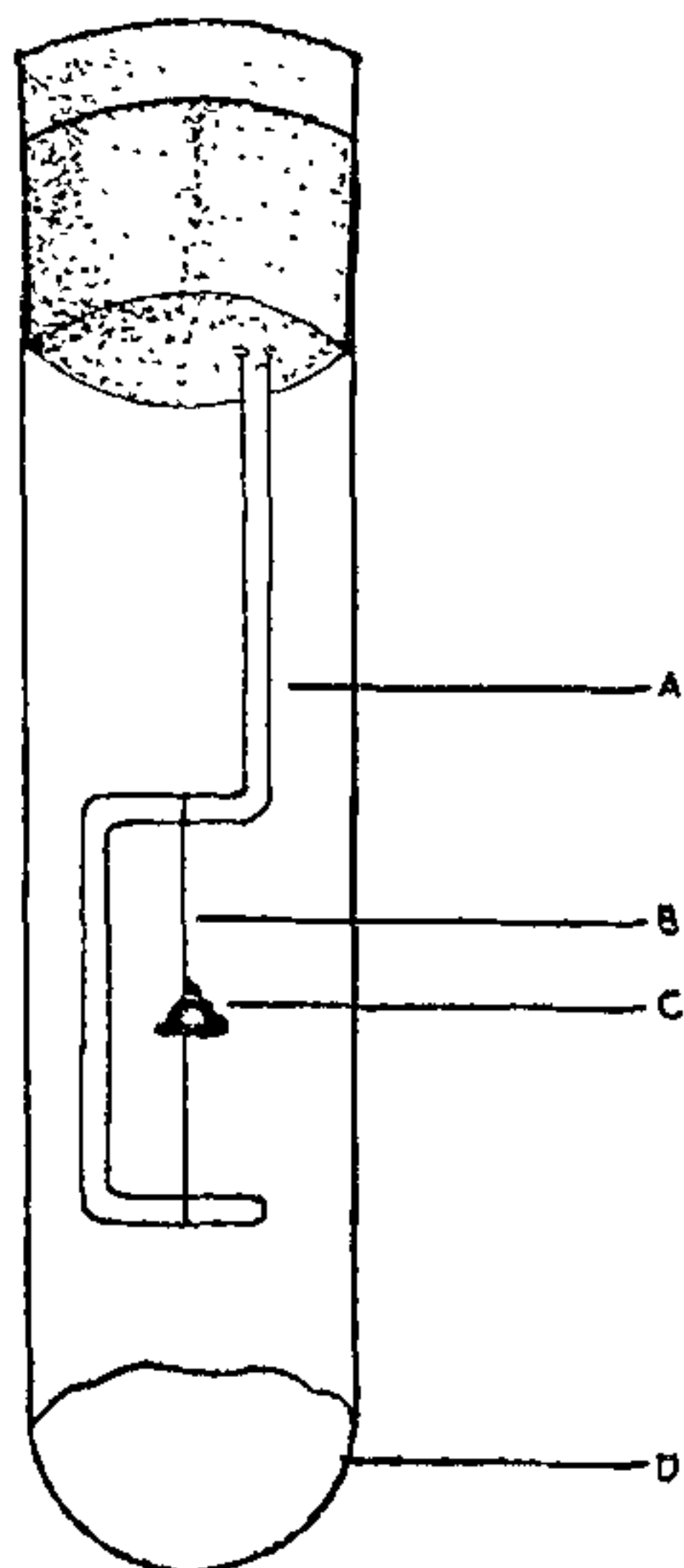


FIG. 1. Growth of test fungus (Keratinophilic) on hair. A, glass rod; B, hair; C, fungal colony; D, moist cotton.

Most of the fungi which attacked keratin containing materials were capable of forming

fronded mycelium and boring hyphae<sup>1</sup>. During the study of keratinophilic fungi from a variety of soils, a large number of fungi which were isolated from the buried hair were found nonkeratinolytic when tested by the above method. This technique can be applied to determine the ability of a fungus to decompose different types of hairs, *i.e.*, human horse, cattle, etc. Keratin decomposing capacity of various keratinophilic fungi can also be assessed by this method.

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#### PROANTHOCYANIDINS IN SEEDS OF THE LEGUMINOUS WEED, *RHYNCHOSIA MINIMA*

DURING seed germination studies on *Rhynchosia minima* we invariably observed the formation of an unusually prominent brownish black halo around each seed sown on moist filter paper discs. We presumed it to be due to leaching by seeds of a large amount of phenolic substances. Therefore, we undertook studies (i) to confirm the presence of phenols in the seed material, (ii) to extract and identify the phenolic substances, and (iii) to find out the effects, if any, of the seed extract on seed germination.

**Experimental Material.**—*Rhynchosia minima* is a leguminous twiner. It bears 1–3 seeds a pod. The seeds are sub-reniform; their average size is 3.35 mm along the longitudinal axis, and 2.5 mm across the widest region. They have a very hard texture; their surface is inconspicuously mottled black and white.

**Seed Germination.**—Seeds were sown on filter paper discs moistened with all-glass distilled water in petri plates. Each petri plate culture contained 9 seeds. Each experiment was replicated thrice with 6 cultures a replicate. All cultures were maintained in light (800–1,000 lux) at 25 ± 2° C for 5 to 10 days, and were periodically irrigated with distilled water. The filter paper discs were not renewed any time during the entire culture period.

Untreated seeds did not germinate; a mere scarification of seeds with sand paper did not also induce germination. Germination occurred only if the