form gave 15 g. of crude mixture of two alkaloids A and B (R, 0·32 and 0·40 respectively on TLC). The residual aqueous solution was acidified to pH 2, reduced with zinc dust, filtered, basified and extracted with chloroform which yielded additional quantities of mixture of A and B.

Attempts to isolate A and B by fractional crystallisation in different solvents failed. The crude mixture (3·5 g.) of A and B was dissolved in chloroform and applied to a column of neutral alumina (250 g. activity grade I) and by graded elutions yielded 1·5 g. B (Benzene), 0·3 g. A + B mixture (Benzene : Chloroform) and 1 g. if A (Chloroform : Ethanol).

Alkaloid A (R_f 0·32) m.p. 195° (lit.[4] monocrotaline 196–197°), picrate m.p. 230° (lit.[4] 231°) and methiodide m.p. 205° (lit.[4] 205°). The alkaloid A was finally proved to be identical with monocrotaline by a mixed m.p. which was undepressed.

Alkaloid B m.f. 136° (lit.[4] crisyatine 137–38°). Elemental Analysis: Found C 61·0; H 7·6; N 4·6%, calculated for $C_{16}H_{23}O_5N$ (Crispatine) C 62·1; H 7·5; N 4·5%.

Alkaline hydrolysis of Alkaloid B was carried out with 2% sodium hydroxide at room temperature for 18 hours. The aqueous solution was extracted with ether to remove unhydrolysed base, acidified with dilute HCl and again extracted with ether. Acid ether extractions on evaporation and crystallisation from benzene gave colourless needles m.p. 133° (lit.[4] 133–34°).

The aqueous residue was evaporated to dryness in a vacuum desiccator. The crystalline residue on extraction with cold alcohol yielded necine-HCl m.p. 160° (after repeated crystallisation from acetone) undepressed on admixture with authentic retronecine hydrochloride.

The alkaloid B was finally proved to be identical with crispatine by comparison of IR spectrum.[4]

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Regional Research Laboratory, O. P. Suri.
Jammu, August 8, 1967. C. K. Atal.

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ON THE CHEMICAL INHIBITORS OF FUNGAL SPORES FROM THE SEEDCOATS OF THREE PLANT SPECIES

The presence of antimicrobial agents on the seedcoats of some plant species was reported by Bowen.[1] Thompson[2] reported that extracts of subterranean clover seedcoats contain a thermostable, water-soluble antibiotic which is inhibitory to a strain of *Rhizobium trifolii* Dangeard. Garber and Houston[3] reported the presence of an inhibitor to *Verticillium albo-atrum* Rein. and Bert. in the seedcoats of both wilt-susceptible and wilt-resistant cotton varieties. The presence of such an inhibitor to the spores of *Helminthosporium oryzæ* Breda de Haan, the fungus causing leaf-spot disease of rice, in the seedcoats of sorghum, ragi and tomato is reported here.

One hundred seeds of each of the three plant species, *viz.*, sorghum (*Sorghum vulgare* Pers.), ragi or finger millet (*Eleusine coracana* Gaertn.) and tomato (*Lycopersicon esculentum* Mill.) were surface sterilized with 0·1% mercuric chloride solution and washed in sterile distilled water. They were added separately to 100 ml. of sterile distilled water contained in 250 ml. Erlenmeyer flasks. The contents were shaken for 6 hr. on a wrist-action shaking machine. Then the suspension was filtered free from seed and other suspensions and the filtrate concentrated *in vacuo* to a final volume of about 5 ml. This concentrate, hereafter referred to as 'seedcoat leachate' was tested for its activity on the spores of *Helminthosporium oryzæ*.

One drop of the spore suspension in sterile distilled water of the fungus, obtained from the growth on oatmeal agar, was placed in the cavity of a microscope slide. To this a drop of the test chemical, *i.e.*, the seedcoat leachate, was added. In the case of checks, additional drop of sterile water was added to the spore suspension in the cavity slide. The slides were incubated in moist chambers at room temperature (22-25° C.) and periodic observations were made. The germination per cent was calculated by examining 100 spores in each microscopic field and taking the average of 10 fields under each treatment. The results are presented in Table I.

There was not only delay in spore germination due to the chemicals, but also there was considerable inhibition of germination and germ-tube growth. The germ-tubes arising from the seedcoat leachate-treated fungal spores were invariably malformed, with characteristic

TABLE I

Effect of seedcoat leachate on the germination of spores of Helminthosporium oryzæ

Source of seed coat leachate	% germination of spores				
	12 hr.	24 hr	48 hr.	72 hr.	96 hr.
Sorghum ..	0	0	0	2*	4*
Ragi ..	0	0	0	14*	12*
Tomato ..	0	0	0	18*	19*
Check (untreated) ..	80	98	..	.	..

* All the germ-tubes of the spores were malformed.

bulbous outgrowths, which got lysed within about 24 hr. after formation. The toxic substance(s) from the three seeds was found to be readily soluble in water, insoluble in most of the organic solvents tested, *viz.*, ethanol, chloroform, carbon tetrachloride, ether, *iso*-propanol and *n*-butanol, and was thermostable and active upto 100° C. Further studies on the antimicrobial activity of the chemical(s), its potency and biochemical properties are in progress.

That the presence of such antifungal substances on the seedcoats might serve as a defence mechanism against seed infection has been suggested by some workers.[4] In the present case the organism concerned is not a pathogen on any of the three plant seeds examined but is inhibited by the chemical(s) produced on the seeds. What would be the action of the chemical(s) on other pathogenic as well as non-pathogenic organisms needs investigation. An understanding of the biochemical and antimicrobial properties of the chemical(s) would help in exploring its usefulness in plant disease control as also in understanding the defence mechanism in the seeds against pathogens.

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SHOOT FORMATION FROM THE CALLUS TISSUE OF HORMONE-TREATED COWPEA LEAVES

COWPEA (*Vigna sinensis* Endl.) has been described as an indicator host of the pathogen which is suspected to cause the root (wilt) disease of coconut palm.[1] During the course of attempts to produce roots in detached trifoliate leaves of cowpea, using IAA, in order to employ them in studies connected with the root (wilt) disease of coconuts, it was observed that shoot primordia also began to develop from the callus tissues of the hormone-treated leaves (Fig. 1). The present note describes this interesting phenomenon.

FIG. 1. Development of shoot primordia from the base.

Two series of different concentrations of IAA, *viz.*, 10^{-2} M, 10^{-3} M, 10^{-4} M, 10^{-5} M, 10^{-6} M and 10^{-7} M were prepared, series I in glass-distilled water and series II in a nutrient solution.[2] Trifoliate leaves of 10-day old cowpea seedlings cut at the base of the petiole just above the region of the axillary bud, were first incubated at room temperature in small vials containing the solutions of series I, with the cut ends dipping in the fluid. Within 5 days of treatment, callus formation commenced at the cut ends of the petiole and roots were initiated. Three days later the hormone-treated leaves were transferred to small vials containing solutions of same concentrations of IAA in nutrient medium (series II) and were maintained as before. Forty-eight hours after they were transferred, shoot primordia developed from the callus tissue of leaves incubated in three concentrations of the solutions of series II, *viz.*, 10^{-5} M, 10^{-6} M and 10^{-7} M. After the initiation of shoots one set of treated leaves was transferred to 250 ml. conical flasks containing nutrient solution alone and another set, to mud pots containing garden soil. In both the sets the primordia developed into