Maltose was superior to sucrose and favoured IAA synthesis from the 4th day of incubation, whereas in sucrose the synthesis reached to its maximum value on the 12th day. Dextrin and starch were almost equal in their efficiency to support IAA synthesis. *G. pentcillioides* could not synthesise IAA at the initial incubation periods, on fructose, surcrose, starch and dextrin. In all carbon sources the synthesising capacity was found to increase with the period of incubation. This may be due to secondary metabolic origin of the substance. Similarly Mahadevan[5] and Narania and Reddy[8] also reported the increasing trend of auxin concentration with the incubation period.

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AN APPROACH TO CONTROL BROWN SPOT OF RICE WITH CHEMICALS KNOWN AS PHYTOALEXIN INDUCERS

IT has been reported earlier from this laboratory that substantial resistance can be induced in rice plants to *Helminthosporium oryzae* Breda de Haan [conidial state of *Cochliobolus miyabeanus* (Ito and Kur.) Drechsler ex Dastur], the brown spot pathogen, by prior inoculation with a mildly virulent isolate or treatment with fluids containing its metabolites[1–3,8]. The occurrence of post-infectionally produced fungitoxic substance of phytoalexin type has been demonstrated for rice plants[1,4,5] and its involvement both in natural host defence[6] and induced host resistance[7,8] has been shown. In the background of such information it was felt that chemicals known to induce phytoalexin production in different plant species[9–13] might be useful in the control of brown spot disease. Results of exploratory studies in this direction are briefly presented here.

Susceptible rice plants (cv. Dharial) and a virulent isolate of *H. oryzae* were the experimental materials. Seventeen chemicals in the nature of heavy metal salts, amino acids, metabolic inhibitors and plant growth regulators, all known phytoalexin inducers[4,9–11], were tested in different experiments conducted as pot or field trials, mostly at dilute concentrations having little effect on spore germination. The pot and field trials were conducted under randomised block design with three replications per treatment. Plants were grown in 20 cm pots. Thirty seeds were sown in each. For field trials, each plot was of 1 sq. metre size. Inoculation of plants with spore suspension (*c.* 5×10^5 conidia/ml) and computation of disease index 4 days later were made following the methods of Sinha and Das[2].

In the initial experiment, all seventeen chemicals were used to spray 3-week-old seedlings in pots 2 days before inoculation. All of them, irrespective of their chemical nature, gave appreciable protection to the seedlings, most effective among them being DL-methionine, indole-3-acetic acid (IAA) and sodium malonate (Table I). Only copper sulphate and

TABLE I

Chemicals (phytoalexin inducers) used to spray rice plants, their more effective concentration, and influence on symptom production resulting from Helminthosporium *infection*

Test chemicals and their more effective conc.		No. of spots per plant*	Disease index per plant*
Water (control)		100·0	100·0
Nickel nitrate	(10^{-5} M)	35·1	31·7
Barium chloride	(10^{-3} M)	54·2	42·3
Ferric chloride	(10^{-4} M)	56·5	43·2
Cadmium chloride	(10^{-4} M)	50·6	49·8
Chromium chloride	(10^{-3} M)	86·1	55·0
Mercuric chloride	(10^{-5} M)	81·3	58·2
Silver nitrate	(10^{-4} M)	74·0	52·7
Copper sulphate	(10^{-5} M)	83·0	56·4
DL–methionine	(10^{-2} M)	37·3	20·3
DL–norleucine	(10^{-2} M)	64·3	34·0
DL–Norvaline	(10^{-2} M)	64·2	49·4
DL–valine	(10^{-2} M)	66·6	53·3
Sodium malonate	(10^{-4} M)	7·1	8·0
Sodium molybdate	(10^{-4} M)	77·4	41·6
Sodium iodoacetate	(10^{-4} M)	52·4	49·4
Sodium fluoride	(10^{-3} M)	72·4	65·9
Indole-3-acetic acid	(10^{-4} M)	27·2	21·7

* Mean of forty-five plants each treatment, fifteen from each pot, expressed as percentage of the control.

silver nitrate showed phytotoxicity at the concentrations used. All compounds reduced the number of lesions. Some of them reduced lesion size also in a significant manner, particularly sodium molybdate, chromium chloride and DL-norleucine.

More promising among the chemicals were further used for seed treatment. Since mercuric fungicides are commonly used in seed treatment, mercuric chloride, though not very effective in spray treatment, was also added to this group. Among the amino acids, only DL-methionine was included. Seeds soaked for 24 hr in solutions of effective concentrations of selected chemicals were sown in pots and also in the field in separate experiments. Pot grown plants were inoculated at the age of 3 weeks, those in the field at 3 or 5 weeks. All the chemicals tested gave good protection to seedlings upto 3 weeks (Table II). In the pot experiment, better protection was recorded with ferric chloride, nickel nitrate, sodium molybdate and DL-methionine. In the field experiment, ferric chloride showed a very strong effect followed by sodium molybdate and mercuric chloride. Plants inoculated at 5-week stage showed a decline in protective effect in all the treatments. The inhibitory effect was mainly on the number of lesions.

TABLE II

Effect of seed soaking for 24 hr in solutions of chemicals, known as phytoalexin inducers, on disease development in rice plants due to Helminthosporium *infection*

Test chemical	Conc.	Mean disease index per plant*		
		Pot Experiment	Field Experiment	
		After 3 weeks	After 3 weeks	After 5 weeks
Water (control)		100·0	100·0	100·0
Nickel nitrate	10^{-5} M	43·0	67·0	90·4
Barium chloride	10^{-3} M	51·9	66·0	90·6
Ferric chloride	10^{-4} M	40·0	11·3	56·5
Cadmium chloride	10^{-4} M	53·4	63·2	62·1
Mercuric chloride	10^{-3} M	64·5	47·2	55·2
Sodium malonate	10^{-4} M	54·1	58·5	68·3
Sodium molybdate	10^{-4} M	43·7	44·3	92·4
Sodium iodoacetate	10^{-4} M	..	62·3	59·3
DL-methionine	10^{-2} M	46·7	52·8	74·5
Indole-3-acetic acid	10^{-4} M	55·1	55·7	55·2

* Mean of forty-five plants each treatment, fifteen from each pot/plot, expressed as percentage of the control.

In another experiment, 3-week-old seedlings were given 24 hr root-dip treatment in solutions of chemicals used for seed treatment at the time of their transplanting from nursery to field plots. Plants were left exposed to natural infection. Assessment of symptoms at intervals showed 35-50% less symptoms in different treatments, except with mercuric chloride and nickel nitrate, after 2 weeks. These two compounds showed less effect. The induced protective effect declined with time in all the treatments ; 12-33% less symptoms were recorded after 5 weeks. Better results were obtained with cadmium chloride, barium chloride, sodium malonate and soldium molybdate. Least effective was mercuric chloride.

Foliage spray gives substantial protection against disease in most of the treatments, but the persistence of induced protective effect at a high level upto 3–5 weeks after seed treatment and to a lesser extent after root-dip treatment seems to be of greater significance. Direct toxic action is ruled cut as a mechanism for induced protection. It is also difficult to assume that induced production of fungitoxic substance as a result of seed treatment can maintain a sufficiently high level in the host tissue even after 3–5 weeks to effectively inhibit the pathogen then. It is tempting, therefore, to speculate that in addition to causing immediate production of fungitoxic substance in the embryo such treatments may also condition it in such a way that new tissues developing from it, upto a period, remain sensitized and produce in response to infection more of fungitoxic substance than usual.

Preliminary study of fungitoxicity in leaf diffusates collected just before and 3 days after inoculation at 3–week stage from plants raised from treated and untreated seeds and grown under field conditions provided some interesting results. Diffusates from uninoculated plants in all the treatments except those with cadmium chloride, DL-methionine and IAA inhibited germ tube growth of *H. oryzae* by 10–50% as compared to that in the diffusate from untreated uninoculated plants. Inoculation itself resulted in strong fungitoxic effect in the diffusate. Diffusates from inoculated plants in all the treatments except those with cadmium chloride and IAA showed stronger fungitoxic effect. The increase in effect was remarkable with sodium malonate and DL-methionine and quite considerable with nickel nitrate and ferric chloride. This would suggest that production of fungitoxic substance in rice leaves resulting from infection with *H. oryzae* is further stimulated on treatment with most of the test chemicals. Such observations and the fact that the induced protective effect involves a reduction not only in the number of lesions but quite often in the lesion size also give some support to the view expressed above on the sensitization of leaf tissue.

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NATURE OF Sg-TOXIN [*SCLEROSPORA GRAMINICOLA* (SACC.) SCHROET] AND ITS ROLE IN SYMPTOMS CAUSATION

PEARL MILLET [*Pennisetum typhoides* (Burm. f) Stapf and Hubb.] is affected by about twenty diseases, amongst which green ear or downy mildew disease caused by *Sclerospora graminicola* (Sacc.) Schroet is important and widespread disease in India. Wani and Rai have extracted toxin (Sg.-toxin) from pearl millet plants affected by *S. graminicola*, studied its nature and its role in the disease symptoms.

Pearl millet plants of HB–3 variety were grown under green house condition and when the plants were of 6–7 cm height inoculated with sporangiospores of *S. graminicola*. The Sg-toxin was extracted from downy mildew affected plants and its toxicity was tested. The host-specificity of Sg-toxin was studied by preparing two fold serial dilutions of crude toxin in aqueous solution, starting from 2·0 p. c. level upto 0·5 p. c. and the test plants were then treated. Eight different crop plants (Table I) representing different families were tested by treating 4·5 cm plant cuttings in different test dilutions of the toxin. These test plants were exposed to illumination and aeration so as to enhance the uptake of the toxin. Observations on the nature of the symptoms developed and time required for development of such symptoms were recorded. The role of Sg-toxin in causation of downy mildew symptoms in pearl millet was studied by soaking the seeds in toxin solution (2·0, 1·0 and 0·5 p. c.) for about 12 hr. Seede soaked in water served as control. These soaked seeds were sown in small pots and grown at room temperaturs ($25 \pm 2°$ C) for about six weeks, The plants were observed regularly for symptoms like germination, dwarfening, chlorosis and wilting of plants.

The antigenic nature of the Sg-toxin was studied by immunising rabbits. One ml of 1·0 p.c. Sg-toxin solution prepared in physiological saline was emulsified with an equal volume of Freund's complete adjuvant and injected intramuscularly twice at an interval of 15 days. Third injection of 1 ml of 1·5 p. c. toxin solution was given intravenously after a gap of 15 days of second injection. Fifteen days after the booster does the blood was collected by puncturing the heart and the serum was collected from the clotted blood. The titer of the antibodies in the serum was determined by micro-precipitin test. The serological relationship between the Sg-toxin and its purified fractions (fractions I and II) obtained by Sephadex gel column chromatography[6] was studied by following the gel diffusion method[2,3]. The immuno-diffusion plates were incubated at 4° C, $25 \pm 2°$ C, and 37° C and observations of precipitation bands were noted.

The results presented in Table I revealed that Sg-toxin was not a host-specific toxin and it wilted cuttings of plants belonging to different families. Toxins isolated from grape leaves affected by *Plasmopara viticola*[3], and coffee leaves by *Hemelia vastatrix* and sunflower leaves by *Puccinia helianthi*[5] have been reported to be non-specific. It was observed that in all the cases wherever toxin caused symptoms the dilution end point of the toxin was almost same but the time required to cause notable symptoms varied considerably. Amongst the various plants tested paddy was found to be the most resistant, followed by greengram and maize in the order of decreasing resistance. The green gram plant cuttings kept in 2 p. c. toxin solution showed curling of leaves after 5 hr and at higher dilutions there was no effect at all. Maize plants showed partial wilting at 0·25 p.c. toxin solution which indicated its resistance to Sg-toxin action. *Sclerospora graminicola* did not infect maize plant in India[5]. The most susceptible plant was found to be tomato. At 0·06 level of the toxin the leaves lost turgidity, drooped down and finally wilted within 210 min. Pearl millet took 300 min. time at 0·06 level to show the symptoms. Because of the non-specific nature of the Sg-toxin it can be considered that the specificity of *S. graminicola* in nature might be due to its nutritional requirements. If a non-host plant