A STUDY OF TOXIC COMPOUND PRODUCED BY SCLEROSPORA GRAMINICOLA

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ABSTRACT : The toxin was isolated from the *Sclerospora graminicola* infected bajra plants called Sg-toxin which was absent in healthy plants. The recovery of Sg crude toxin from infected bajra plants was about 0.5 per cent on dry weight basis. The Sg-toxin was toxic to bajra and tomato plant cuttings but tomato plant was more sensitive. The Sg-toxin was toxic up to 0.06 per cent level of dilution. It inhibited the germination of seeds and decreased the plumule length particularly in case of bajra. It did not affect the growth of any of the 23 microorganisms tested. The Sg-toxin was a vivo-toxin and non specific in nature.

Downy mildew or green ear disease of pearl millet is one of the most important and widespread diseases amongst the twenty dieseases reported on this crop. The causal agent of this disease *Sclerospora graminicola* (Sacc.) Schroet. is an obligate parasite on its host. Even though, this disease causes a devastating damage to bajra crop. till now there is no proper control measure except using resistant varieties because exact mechanism of causation of this disease is not well understood. Rai (1977) reported a toxic compound produced by *Plasmopara viticola* from infected grape leaves. Similarly, Wani and Rai (1978) have reported toxic compounds from *Hemileia vastatrix* infected coffee leaves and *Puccinia helianthi* infected sunflower leaves. This study was undertaken to find out whether any toxic substance is produced in pearl millet plants infected by *S. graminicola* and to study its biological properties.

MATERIAL AND METHODS : Bajra plants of H. B.—3 variety were grown in green house. Six to seven cm tall bajra plants were inoculated early in the morning with sporangiospores of *S. graminicola* collected from infected leaves, incubated in moist chamber overnight. The bajra plants showing typical symptoms of the disease i.e., chlorosis and yellowing (and green ear in rare case) were collected, air dried, powdered and extracted by following the procedure outlined in Fig. 1. Similarly, the control bajra plants without sporangiospore inoculation were extracted and the compounds extracted from both types were assayed for toxicity by using tomato and bajra plant cuttings. Two fold dilutions starting from 2.00 per cent level were prepared by dissolving substances obtained from healthy and diseased bajra plants in distilled water. Young 10 day old cuttings of HB—3 bajra variety and tomato cuttings were placed in serially diluted samples. The time required to develop visible symptoms in plant cuttings was noted.

Effect of toxin on seed germination, and plumule growth was tested by using seeds of eight plant species. Healthy seeds, were surface sterilized by using

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0.1 per cent mercuric chloride, washed 6-7 times in sterile water and soaked in dilutions of 2.0 per cent, 1.0 per cent and 0.5 per cent toxin for 12 hr. Seeds soaked in sterile distilled water and 5 per cent normal plant extract served as control. The soaked seeds were placed in sterile moist filter paper discs in sterilized petriplates. The plates were incubated at room temperature for 3-4 days and sterile water was added as needed. The observations of germinating seeds particularly germination percentage and plumule lengths were recorded.

To study the effect of toxin on the growth of microorganisms 23 different microbial cultures representing different groups viz., actinomycetes, algae, bacteria and fungi were used. The following cultures were used : *Aerobacter aerogens, Azotobacter chroococcum, Bacillus subtilis, Escherichia coli, Klebsiella* sp., *Nocardia* sp., *Pseudomonas solanacearum, Rhizobium* sp., *Sarcinia lutea, Streptococcus aureus, Actinomyces* sp., *Streptomyces* sp., *Saccharomyces cereviseae, Chlorella* sp., *Alternaria* sp., *Aspergillus* sp., *Chaetomium* sp., *Fusarium* sp., *Ganoderma* sp., *Helminthosporium oryzae, H. sacchari, Phytophthora arecae* and *Pyricularia* sp. For actinomycetes and bacterial cultures standard filter paper disc method for algal and fungal cultures antibiotic cup assay method were followed. Different toxin concentrations viz., 4 per cent, 2 per cent and 1 per cent were tested for its antimicrobial activity. The tests were replicated four times. Observations on the presence or absence of an inhibition zone and its diameter, if present, were recorded.

RESULTS : The toxin was isolated from the powdered, downy mildew infected bajra plants by the method summarized in Fig. 1. The substance obtained after passing through appropriate Dowex resin columns was referred as Sg-crude toxin. The recovery of Sg-crude toxin was estimated as shown in Fig. 2. About 98.25 per cent material was lost as solid waste during the first step of extraction. The precipitated compound constituted only 1.75 per cent of the total plant material on dry weight basis. The recovery of Sg-crude toxin from infected bajra plants was only 0.49 per cent and 99.51 per cent material was lost during the first two steps of Sg-toxin purification. These results indicated that Sg-crude toxin was present in infected bajra plants but in traces.

The preliminary studies showed that tomato plants displayed wilting symptoms compared to bajra plants. The least concentration required to cause wilting was 0.06 per cent in 3.5 hr. The fraction extracted from healthy bajra plants did not cause wilting or any symptoms even at 2.00 per cent level. At the beginning, leaves of the plants in toxin solution lost the turgidity and then curling of leaves from tip towards petiole started. The plants started drooping down and showed wilting symptoms. The stem of the wilted plants were flattened, fragile and sunken. None of these symptoms developed in control plants. There was an inverse relationship between toxin concentration and time required for wilting up to a concentration of 0.125 per cent. At low concentrations, the time required for wilting increased. However, at 0.06 per cent and higher dilution, no such inverse relationship between toxin quantity and time to cause symptoms existed.

The results of the experiment on effect of toxin on seed germination, radicle and plumule lengths (Table 1) indicated that Sg-toxin affected seed germination. The direct relationship between toxin concentration and inhibition of seed germination was obtained in case of bajra and ragi seeds. At 2.0 per cent level of the toxin bajra and ragi seed germination was inhibited by 27 and 12 per cent respectively. At one per cent level the inhibition of germination in bajra and ragi was 13 and 6 per cent respectively, as shown in Table 1. This clearly indicated that there was an

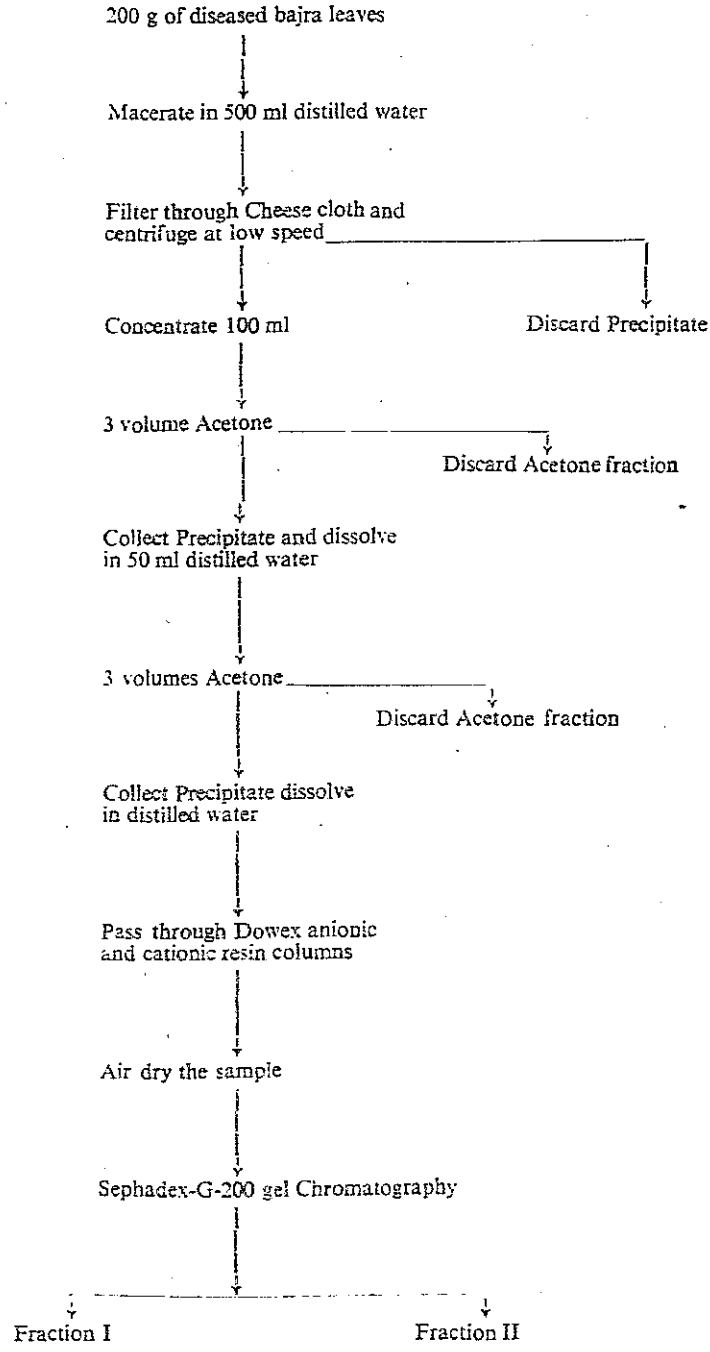

Fig. 1. Flow sheet summarizing the procedure used to purify *Sclerospora graminicola* toxin.

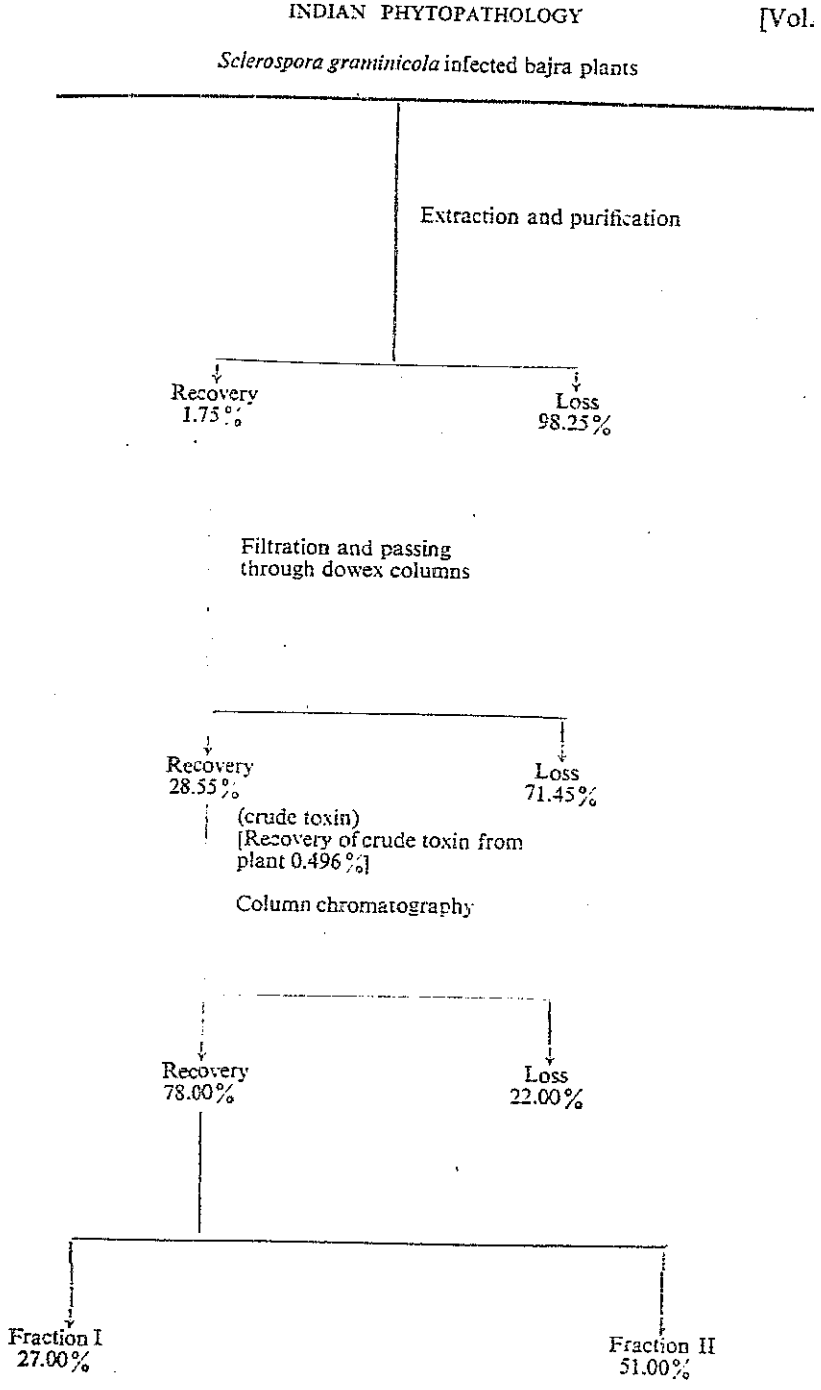

Fig. 2. Flow sheet summarizing the percentage recovery of *Sclerospora graminicola* toxin during its extraction and purification processes.

inverse linear relationship between the toxin concentration and seed germination, inhibition of crops in particular of bajra and ragi. In other cases, the germination was affected by Sg-toxin to a considerable extent but the relation was not linear (Table 1). The radicle length of various germinating seeds showed a varied response. In majority of the cases the Sg-toxin treatment of seeds before germination caused increased radicle lengths. Among the 8 crop seeds studied, increased radicle length occured in 5 crop seeds viz., bajra, jowar, wheat, green gram and the fox tail millet (*Setaria italica*). A direct relationship existed between the toxin concentrations and the increased radicle length. The radicle growth was inhibited in case of ragi, tomato and blackgram to some extent (Table 1). The data in Table 1 also indicated that the plumule length decreased as the toxin concentration increased. There was no direct relationship between the increased toxin concentration and the reduced plumule length. The plumule length of black gram slightly increased. Admittedly Sg-toxin adversely affected the plumule and not the radicle growth.

TABLE 1 : Effect of *Sclerospora graminicola* toxin on germination and plumule length of different seeds

Name of the crop	Test	Control	Toxin concentration (in per cent)		
			0.5	1.00	2.00
Bajra	A*	100.00	97.00	87.00	73.00
	B	2.57	2.40	2.30	2.40
Ragi	A	100.00	98.00	94.00	88.00
	B	2.35	2.28	1.97	1.97
Jowar	A	73.00	66.00	66.00	57.00
	B	2.47	2.34	2.02	1.69
Wheat	A	88.00	88.00	88.00	84.00
	B	3.35	3.30	3.20	2.25
Tomato	A	86.00	84.00	80.00	66.00
	B	3.50	3.42	3.40	3.33
Green gram	A	100.00	76.00	72.00	60.00
	B	3.17	2.05	1.67	1.53
Black gram	A	90.00	88.00	84.00	80.00
	B	0.90	0.95	1.00	1.00
Setaria italica	A	94.00	80.00	76.00	74.00
	B	2.20	1.52	1.68	2.06

*A = Germination percentage
B = Plumule length (cm)

The Sg-toxin did not affect the growth of any of the 23 microrganisms, belonging to algae, actinomycetes, bacteria and fungi even at 4 per cent concentration.

DISCUSSION : A toxic fraction, which occurred in traces was isolated from the infected bajra plants. Millard and Scott (1955) reported a toxin from powdery

mildew infected bajra plants. Rai (1977) reported the production of a non-specific toxic compound in grape leaves infected by *Plasmopara viticola*. Wani and Rai (1978) have extracted toxic compounds from coffee and sunflower plant leaves infected with *Hemileia vastatrix* and *Puccinia helianthi* respectively. About 0.50 per cent toxic compound was present in infected bajra plant (Fig. 2). Detection of the toxin becomes a problem because of its occurrence in minute amounts. Toxicity of the compound was tested by using tomato and bajra plant cuttings as suggested by various workers (Hodgson *et al.*, 1947, 1949, Rai, 1977, and Wani and Rai, 1978). Bioassay studies revealed that the dilution end point of the Sg-toxin was 0.06 per cent. The recovery of the toxin from infected plants and its dilution end point suggested that *S. graminicola* produced a large amount of the toxic compound in infected plants compared to that necessary to cause disease symptoms. *S. graminicola* produced sufficient amount of toxic compound in infected plants to cause disease symptoms and such a toxic compound could not be isolated from healthy plants. These results fulfilled the criteria for vivotoxin suggested by Dimond and Waggoner (1953). The symptoms were caused by 2.0 per cent toxin solution in 30 min. which indicated that the toxin was quite potent and highly toxic in activity.

The results from Table 1 indicated that bajra and ragi seeds were affected more by Sg-toxin compared to other seeds and this might be due to the partial specificity of Sg-toxin. *S. graminicola* affects bajra and can be grown on ragi callus (Safeeulla, 1976). It appears reasonable to expect that seeds absorb Sg-toxin in soil and loose vaiability. This is of biological interest because *S. graminicola* is cosidered as a soil borne pathogen, if germinating oospore produce the toxic principle, the toxin might be starting its activity from the very early stage. Natour (1957) reported a toxin from germinating uredospores of *Puccinia graminis* var. *tritici*. The inhibition of seed germination might be due to the affect of toxin on the embryo of the seeds. It is known that *S. graminicola* affects mainly leaves and shoot portion of the plants. The increased redicle length might be due to some growth promoting properties of Sg-toxin whose effect was confined to the radicle. It needs further confirmation and studies for concluding the nature of the substances involved. Bajra plants infected by *S. graminicola* in later stage produce the green earhead where ovaries will be converted into leaf like structures. This also suggests the association of growth promoting substances with downy mildew. The growth regulators were reported to the involved in plant diseases caused by bacteria and fungi (Thimann and Sachs, 1966 and Wani *et al.*, 1977). In general, toxins from phytopathogenic fungi are reported to inhibit the radicle length of germinating seeds (Guenzi and McCalla, 1964 and Scheffer and Ullstrup, 1965). However, Kuo and Scheffer (1967) and Kuo *et al.*, (1970) reported that dilute solution of *Helminthosporium carbonum* toxin stimulated root growth of corn seedlings. Downy mildew affected plants are generally stunted. The reduced plumule length supported and clarified the reason for the stunted growth of such infected bajra plants. The Sg-toxin did not affect the growth of any of the microorganisms tested. It suggested that Sg-toxin was group specific if not host-specific.

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