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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 ± 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 ± 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

TABLE I

Host-specificity of Sclerospore graminicola *toxin*

Name of the crop	Toxicity	Dilution end point	Time (min)	Remarks
Bajra (*Pennisetum typhoides*)	+	0·06	300	At the beginning leaves lost turgidity, curling and drooping of leaves took place and at higher concentrations leaves wilted completely and dried.
Tomato (*Lycopersicon esculentum*)	+	0·06	·210	Leaves lost turgidity, cuttings drooped down and finally wilted.
Black gram (*Phaseeolus mungo*)	+	0·06	360	Leaves lost turgidity and cuttings drooped down.
Green gram (*Phaseolus aureous*)	±	..	..	In 2·0 p.c. concentration curling of leaves was observed after five hours only.
Wheat (*Triticum vulgare*)	+	0·06	1080	Leaves lost turgidity.
Ragi (*Eleucine coracana*)	+	0·06	360	Wilting of cuttings took place.
Maize (*Zea mays*)	±	0·25	480	Partial wilting was observed, but almost no reaction to toxin.
Paddy *Oryza sativa*)	–	..	..	No reaction.

could provide with the nutrients required for the fungus growth, then it might attack some of the non-host plants also. *S. graminicola* has been grown on ragi callus which is not a host for the pathogen[5]. Even after removing the callus from the medium, the fungus survived for some time in the absence of callus. These findings support the nutritional hypothesis for host-specificity of *S. graminicola* in nature proposed on the basis of Sg-toxin studies.

The reduced germination percentage of pearl millet seeds due to soaking in Sg-toxin was recorded. About 40 p.c. germination was reduced when the seeds were soaked in 2 p.c. toxin and at 1 and 0·5 p.c. level it was reduced from 5 to 12 p.c. The notable differences amongst the treatments were visible in two weeks. The symptoms like chlorosis, yellowing and curling of leaves from tip towards base were first observed in 1·0 p.c. toxin treated seeds. One or two days later leaves became fragile and finally wilted. These symptoms shown by the toxin treated plants are some of the major symptoms in case of downy mildew of bajra. At 0·5 p.c. toxin treatment similar symptoms but at lesser intensity were noted. The pearl millet plants grown from seeds soaked in Sg-toxin mimicked some of the symptoms of the downy mildew disease. These findings have also been supported by histochemical studies[7].

The Sg-toxin could be isolated only from the pearl millet plants affected by *S. graminicola* and not from the healthy plants[7]. Sg-toxin is glycopeptide in nature and it has been chemically characterised[7]. When the Sg-toxin was introduced in healthy pearl millet seeds, it produced most of the symptoms of downy mildew disease of pearl millet. These studies indicated that Sg-toxin is a 'vivotoxin' as stated by Dimond[1] and non-host-specific but group specific as it did not affect any of the microorganisms tested[7]. This is the first report revealing the nature of the toxin associated with an obligate plant parasite.

The titer of the antibodies aganist Sg-toxin in the antiserum was found to be 1 : 1280 by microprecipitin test. It revealed that Sg-toxin was antigenic in nature. The results of the immuno diffusion experiment indicated that Sg-toxin and its purified fractions I and II were serologically related. The precipitation bands did not cross each other in any case. An optimum temperature for incubating the immuno diffusion plates was found to be 4°C wherein the bands appeared within 24 hr. At 25 ± 2°C the time required for appearance of the precipitation bands was 48 hr and at 37° C the bands did not appear even after 48 hr.

The financial help by Bombay University through Global Impacts of Applied Microbiology Fellowship and Indian Council of Agricultural Research through United Nations Development Program funds is duly acknowledged.

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April 28, 1979.

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THE INFLUENCE OF PRE-SOWING SOAKING WITH MINERAL SOLUTIONS ON THE SEEDLING GROWTH IN METHI (*TRIGONELLA FOENUM-GRAECUM* L.) VAR. "PUSA EARLY BUNCHING"

PRE-SOWING soaking of seeds in mineral solutions is reported to hasten seed germination in several cultivated plants[4], increase seedling length in pumpkin[5], improve vegetative growth[4] and dry matter production[1], and increase final yield in carrot[1]. Most of the work done on the pre-sowing seed treatment with minerals has been reviewed by Heydecker and Coolbear[2]. Methi (*Trigonella foenum-graecum* L.), a common leafy vegetable, is also known for the medicinal value of the seeds. Studies were undertaken on the response of methi to pre-sowing seed soaking with minerals.

Seed of methi (*Trigonella foenum-graecum* L.) Var. 'Pusa Early Bunching' obtained from the National Seeds Corporation, Bangalore, were soaked in 0·01%, 0·1% and 1% solutions each of sulphates of ammonium, copper, manganese and zinc and of boric acid. The treatment consisted of soaking for 4 h in the respective media followed by air drying to their original weight (for 40 h). The treatment was given at ambient temperature ranging from 25° C to 27° C with a light intensity of 1200 Lux (fluorescent lamps). The treated seeds were set for germination in 10 cm. dia. Petri-dishes lined with moist blotters in 5 replicates of 10 seeds each. Unsoaked and the water soaked seeds were included in the experiment for comparison. These seeds were set to germinate under 24 h cycles of 10 h photoperiod of 1100 Lux at 27 ± 1° C. The experiment was repeated thrice. Dry weights of 96 h old seedlings were recorded.

The seedlings were also raised in soil in polythene bags employing the seeds soaked in boric acid (0·01%), copper sulphate (0·1%), manganese sulphate (0·01 and 0·1%) and zinc sulphate (0·1%). The 10 day old seedlings were employed for determining the dry matter production. Statistically analysed data are presented in Figs. 1 and 2.

Loo and Tang[4] have reported that seed soaking with manganese sulphate accelerated seed germination in several cultivated plants. In the present study manganese sulphate hastened the germination only by 4 to 6 h. Soaking treatments with boric acid (0·01 to 0·03%) and sulphates of manganese (0·1 to 0·4%) and zinc (0·05 to 0·1%) are reported to increase germination per cent in pea, cabbage and tomato[3]. But in methi none of these salts could change the germination per cent which remained constant at 85% upto 1% of the salt concentrations.

It is seen from the data in Fig. 1, that soaking in water brought about 4·9% and 26·3% increase in dry weight of shoot and root respectively, of the 96 h old seedlings of methi. Soaking with minerals gave a further increase of 1 to 20% in shoot and 9 to 63% in root dry weight over and above the water soaked ones. Manganese sulphate at 0·1% was the most effective being followed by 0·1% of zinc sulphate and copper sulphate and 0·01% of boric acid and ammonium sulphate.

Data in Fig. 2 show that the beneficial effect of minerals observed in 96 h old seedlings persists even in the 10 day old seedlings, although there was a decline in the magnitude of the beneficial effects in the 10 day old seedlings. The dilution effect was the greatest with 0·1% copper sulphate (82·1%) and lowest with 0·01% manganese sulphate (59·5%). Even though 0·1% manganese sulphate showed 73·5% decrease in the beneficial effect, it was the most effective among the treatments, even at 10 day old seedling stage. Pre-sowing soaking of seeds in manganese and boron solutions is reported to be useful in increasing the dry matter of carrot seedlings[1]. Krauja[3] has also shown that seed treatment with 0·01 to 0·03% of boric acid, 0·05 to 0·1% of zinc sulphate and 0·1 to 0·4% of manganese sulphate will increase the dry matter content