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Monitoring of heavy metal (zinc sulphate) toxicity by using pollen as Indicators - Pollen of *Vigna unguiculata*: Further Evidence of a Criticism of Banerji and Gangulee (1937), Dharurkar (1971 - Ph.D. Thesis), Nair, Nambudiri and Thomas (1973), Berg (1973), Brandt (1974), Vick and Bevan (1976), Rasmussan (1977), Navara, Horvath and Kaleta (1978), Mhatre (1980 - Ph.D. Thesis), Mhatre, Chaphekar, Ramani Rao, Patil, Haldar (1980), Shetye (1982 - Ph.D. Thesis) and Giridhar (1984 - Ph.D. Thesis) - A Critical Review

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Abstract

Zinc sulphate affected the germination of seed of *Vigna unguiculata* (L.) Walp. The treatment of 0.1 mg/ml of zinc sulphate showed the adverse effect on the phenology. It caused decrease in the fertility of pollen with an increase in the concentrations. This proves the gametocidic behaviour of the heavy metal. Though it affected the fertility of pollen, however, none of the concentration could bring down the fertility to zero percent. Potentiality of the germinability of pollen was noted in all the 4 series *i.e.* F, F-24, F-48, F-72 investigated. The heavy metal stimulated the germination of pollen of F, F-24 and F-48 series. All the concentrations of zinc sulphate inhibited the germination of pollen of F-72 series as well as the pollen tube growth of all the 4 series. The present investigation also shows that pollen germination and tube elongation are two different processes differing in their sensitivity to different concentrations of the heavy metal. It is also confirmed that the pollen development and activity are more sensitive indicators of adverse factors in the botanical environment and the use of an entire vascular plant as an indicator of pollution is a very crude method and rather a wrong choice.

Keywords :- *Genetics and Plant Breeding, Palynology, Crop Physiology, Heavy Metals*

Introduction

Zinc increases the fibre strength of cotton. It has been also seen that if cotton seeds are soaked in a zinc before sowing or if zinc applied to the roots of cotton seedlings, the zinc is translocated to actively growing parts of the plant. However, an excess of zinc in soil suppresses phosphorus uptake by plants and can cause leaf chlorosis (Bertrand and Wolf, 1961).

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Materials and Method

Certified seeds of *Vigna unguiculata* (L.) Walp. Var Pusabarsati (cowpea) of Delhi were obtained from the authorized dealers from which healthy seeds were selected. To study the effect of zinc sulphate, 20 seeds of *V. unguiculata* were sown in white-transparent polythene bags (35x25 cm) containing garden soil and each bag was treated with a 500 ml of different concentrations (0.001, 0.01, 0.1, 1, 10, 100, 1000 mg/ml) zinc sulphate immediately after sowing the seeds. The treatment was given on every alternate day till the life cycle of the crop.

A set of control plants was also grown simultaneously with only water in the same quantity as the treated sets. Excess plants were removed after 15 days of sowing leaving the identical and healthy 5 plants in each bag. There were 10 replicates of each treatment. The observations regarding mortlity, morphology, anatomy, phenology *etc.* were recorded on every alternate day. After 4 weeks of an uniform flowering, successive flowers (*viz.* F, F-24, F-48, F-72 series *i.e.* open flowers and the flower buds which require 24, 48, 72 hours to open respectively) were plucked t the same time after the dehiscence of anthers (in open flowers). Pollen viability was tested by using 2,3,5-Triphenyl tetrazolium chloride (Hauser and Morrison, 1964). To find out the germination potential of pollen in the bud stage of floral development, the flower buds of various sizes marking the various stages of development and the open flowers were plucked at the same time, after the dehiscence of the anthers (in open flowers). Germination of pollen grins of successive flowers was studied by standing-drop technique in an optimum concentrations of sucrose as: 10%sucrose for F-24 and F-48 series, 20% sucrose for F-72 series and 50% sucrose for F series. The cultures were then transferred to a moist filtered chamber, stored at room temperature (27-31°C) having RH of 53% and in diffuse laboratory light. The experience were run in triplicate and average results were recorded. Observation were made by 24 hours after incubation. For each experiment a random count of 100 grains was made (from different fields of the slide) to determine the pollen viability and germination. For measurement of length of pollen tubes, 50 tubes were selected randomly and measured at a magnification of 100x. The data obtained was statically analyzed applying 't' test.

Results and Discussion

Zinc sulphate affected the germination of seed of *Vigna unguiculata.* The treatment of the lowest concentration of the heavy metal showed 38.00% seed germination against 50.00% in control, while only 3.50% was noted with the treatment of 1000 mg/ml. Zinc sulphate could not cause cent percent mortality. The treatment of 1 mg/ml of zinc sulphate showed the adverse effect on the phenology, as the result of which F-72 series stopped further flowering after 29 days of their initiation. The treatment of 100 mg/ml inhibited an initiation of flowering of all the series except for F series after 31 days of their initiation, while further an initiation of all the series had been completely suppressed after 32 days of their onset.

Zinc sulphate caused decrease in the fertility of pollen of *Vigna unguiculate* with an increase in the concentrations (Table 1). This proves the gametocidic behaviour of the heavy metal. Salgare and Suwarna Gawde (1991) recorded the gametocidic effect of the heavy metal on the leguminous crop. It should be noted that though zinc sulphate affected the fertility of pollen of *V. unguiculate*, however, it could not bring down to zero percent (Table 1).

As a rule the percentage of pollen germination is always less than the pollen viability (Table 1). However, Banerji and Gangulee (1937) and Dharurkar (1971) reported higher percentage of pollen germination than the pollen viability in *Eichhornia crassipes.* The claim of Banerji and Gangulee (1937) and Dharurkar (1971) is challenged by Salgare (1986a, 95, 2000b, 2006a, 2006i, 2006j, 2006k, 2006m, 2007a, 2007b, 2007d, 2007e, 2007f, 2007h, 2007i) who stated that the observations of Banerji and Gangulee (1937) and Dharurkar (1971) are exaggerating.

Potentiality of the germinability of pollen was noted in all the 4 series *i.e.* F, F-24, F-48, F-72 investigated (Tables 2 and 3). The treatment of 0.001, 0.01, 0.1 mg/ml concentrations of the heavy metal stimulated the germination of pollen of F and F-24 series. The treatment of all the concentrations of zinc sulphate inhibited the germination of pollen of F-48 and F-72

Table 1: Effect of zinc sulphate (supplied through water) on the fertility of pollen of successive flowers of *Vigna unguiculata*

Conc.	Successive flowers							
	F		F-24		F-48		F-72	
	Tin%	DFC%	Tin%	DFC%	Tin%	DFC%	Tin%	DFC%
0.001	66.20±1.98	-14.03	63.20±3.05	-17.92	57.00±3.47	-25.97	53.20±3.02	-30.91
0.01	63.80±2.19	-17.14	61.60±1.39	-20.00	53.20±5.98	930.91	50.60±3.42	-34.29
0.1	61.20±2.79	-20.26	56.80±2.17	-26.23	49.20±2.17	-36.10	44.00±3.08	-42.86
1.0	60.20±4.55	-21.82	56.20±2.08	-27.01	48.40±2.37	-37.14	Nf	Nf
10.0	57.80±2.39	-24.94	55.00±4.50	-28.57	Nf	Nf	Nf	Nf
100.0	56.00±2.55	-27.27	Nf	Nf	Nf	Nf	Nf	Nf
1000.0	APD	APD	APD	APD	APD	APD	APD	APD

Note: APD, all plants died; Conc,. Concentrations of heavy metal in mg/ml; DFC, difference from control; Nf, no flowering; T, pollen viability in treated sets. Values given are mean ± SE of 500 (Tested 4 weeks after initiation of flowering)

Table 2: Effect of zinc sulphate (supplied through water) on the germination of pollen of successive flowers of *Vigna unguiculata*

Conc.	Successive flowers							
	F		F-24		F-48		F-72	
	Tin%	DFC%	Tin%	DFC%	Tin%	DFC%	Tin%	DFC%
0.001	24.00±2.82	+60.00	9.40±1.86	+56.67	5.00±1.09	-13.79	3.20±0.86	-33.33
0.01	20.80±1.85	+38.67	8.00±1.89	+33.33	4.20±0.86	-27.59	2.00±0.55	-558.33
0.1	17.80±2.19	+18.67	7.00±2.02	+16.67	3.40±0.68	-41.38	Ng	Ng
1.0	7.80±1.28	-48.00	3.00±0.89	-50.00	2.00±0.45	-65.52	Nf	Nf
10.0	4.20±1.32	-72.00	1.00±0.32	-83.33	Nf	Nf	Nf	Nf
100.0	2.00±0.71	-86.67	Nf	Nf	Nf	Nf	Nf	Nf
1000.0	APD	APD	APD	APD	APD	APD	APD	APD

Note: APD, all plants died; Conc,. Concentrations of heavy metal in mg/ml; DFC, difference from control; Ng, no pollen germinationNf, no flowering; T, pollen viability in treated sets.
Values given are mean ± SE of 500 (Tested 4 weeks after initiation of flowering)

series (Table 2). The treatment of all the concentrations of the heavy metal inhibited the pollen tube growth of all the 4 series of *V. unguiculata* (Table 3).

The present investigation also shows that pollen germination and tube elongation are two different processes differing in their sensitivity to different concentrations of the chemical (Tables 2 and 3). This was also pointed out earlier by the author (1986, 2006c). However, Nair, Nambudiri and Thomas

Table 3: Effect of zinc sulphate (supplied through water) on the pollen tube growth of successive flowers of *Vigna unguiculata*.

Conc.	Successive flowers							
	F		F-24		F-48		F-72	
	Tin (mm)	DFC%	Tin (mm)	DFC%	Tin (mm)	DFC%	Tin (mm)	DFC%
0.001	158.00±15.91	-21.78	194.00±22.89	-19.17	110.00±16.-99	-26.67	32.00±3.74	-36.00
0.01	146.00±15.97	-27.72	184.00±26.52	-23.33	96.00±18.02	-36.00	30.00±7.06	-40.00
0.1	136.00±34.38	-32.67	170.00±18.13	-29.17	68.00±10.66	-54.67	Ng	Ng
1.0	128.00±15.27	-36.63	160.00±14.12	-33.33	40.00±10.47	-73.33	Nf	Nf
10.0	122.00±19.81	-39.60	150.00±18.68	-37.50	Nf	Nf	Nf	Nf
100.0	70.00±7.06	-65.35	Nf	Nf	Nf	Nf	Nf	Nf
1000.0	APD	APD	APD	APD	APD	APD	APD	APD

Note: APD, all plants died; Conc,. Concentrations of heavy metal in mg/ml; DFC, difference from control; Ng, no pollen germinationNf, no flowering; Tinn, pollen tube length in mm in treated sets
Values given are mean ± SE of 50 (Tested 4 weeks after initiation of flowering)

(1973) stated that it has been significant that the optimum percentage of germination and tube length were attained in the same growth medium. Present work (Tables 2 and 3) as well as previous extensive work of Salgare (1979, 1983, 1986b, 2004, 2005a, 2005c, 2006b, 2006 g, 2006m, 2007h, 2007i), Salgare and Bindu (2002, 2005), Salgare and Tessy Mol Antony (2005a, 2005b) and Salgare and Joshi (2007) it could be concluded that the observations of Nair, Nambudiri and Thomas (1973) are superficial and misleading.

Thus it is confirmed that the pollen development and activity are more sensitive indicators of adverse factors in the botanical environment and the use of an entire vascular plant (Berg, 1973; Brandt, 1974; Vick and Bevan, 1976; Rasmussan, 1977; Navara, Horvath and Kaleta, 1978; Mhatre, 1980-Ph.D.Thesis; Mhatre, Chaphekar, Ramani Rao, Patil, Haldar, 1980; Shetye, 1982-Ph.D.Thesis and Giridhar, 1984-Ph.D.Thesis) as an indicator of pollution is a very crude method and rather a wrong choice. There is no evidence of any entire vascular plant exhibiting this much degree of sensitivity. This is confirmed in the present critical review (Table. 1) as well as by the previous extensive work of Salgare (1984, 85a-c, 86c-d, 2000a, 01a-b, 05b, d-e, 06a, c-f, h, j-o, 07a-k), Salgare and Theresa Sebastian (1986, 2006), Salgare and Phunguskar (2000), Salgare and Sanju Singh (2002, 06a-b) and Salgare and Sanchita Pathak (2005).

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