A REVIEW:

Evaluation of Heavy Metal (copper sulphate - supplied through water) as Male Gametocide on *Vigna unguiculata* (L.) Walp. and Salgare's Method of Plant Breeding: Further Evidence of a Criticism of Banerji and Gangulee (1937), Dharurkar (1971 - Ph.D. Thesis), Nair, Nambudiri, 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)

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Copper is essential to life and health, its deficiency and excess both cause adverse effects.

To study the effect of zinc sulphate, 20 seeds of *Vigna unguiculata* (L.) Walp. var Pusabarsati (cowpea) of Delhi 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) copper 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 mortality, morphology, anatomy, phenology *etc.* were recorded on every alternate day. After one week of 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 at the same time after the dehiscence of anthers (in open flowers). Viability of pollen was tested by using 2,3,5-Triphenyl tetrazolium chloride (Hauser and Morrison, 1964). 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 (24-30°C) having RH of 54% and in diffuse laboratory light. The experiences were run in triplicate and average results were recorded. Observations were made by 24 hours after incubation. For each experiment a random count of 100 grains was made 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.

Copper sulphate affected the germination of seed of *V. unguiculata*. The treatment of the lowest concentration of the heavy metal showed 50.00% seed germination equal to that in control, while only 10.50% was noted with the treatment of 1000 mg/ml. Copper sulphate caused cent percent mortality with 1000 mg/ml treatment. The treatment of 100 mg/ml of copper sulphate showed the adverse effect on the phenology, as the result of which stopped further flowering after one week of their initiation.

Copper sulphate caused decrease in the fertility of pollen of *V. unguiculata* (Table 1). This proves the gametocidic behaviour of the heavy metal. Though copper sulphate affected the fertility of pollen of *V. unguiculata*, however, it could not bring down to zero percent which is essential for successful plant breeding program (Table 1). Potentiality of the germinability of pollen was noted in all the 4 series (Tables 2 and 3).

Banerji and Gangulee (1937) and

Table 1 : Effect of Heavy Metal (copper sulphate - supplied through water) on pollen fertility of successive flowers of *Vigna unguiculata*

(Tested 3 weeks after initiation of flowering)
(Values given are mean ± SE of 500)
% Pollen fertility
Successive flowers

	F		F-24		F-48		F-72	
Conc.	%PF	%DFC	%PF	%DFC	%PF	%DFC	%PF	%DFC
0.001	76.40±4.12	-06.14	74.00±2.88	-09.09	71.60±1.69	-12.04	68.40±3.1766.0	-15.97
00.01	72.20±2.59	-11.30	70.00±3.41	-14.00	66.80±1.43	-17.94	0±1.6961.80±1.	-18.92
000.1	70.00±3.85	-14.00	67.40±2.06	-17.19	64.20±2.41	-21.13	74	-24.08
0001	67.60±3.74	-16.95	64.20±2.06	-23.13	61.80±2.26	-24.08	59.40±3.00	-27.03
0010	66.80±2.45	-17.94	63.40±1.72	-22.11	58.60±4.02	-28.01	54.40±2.90	-33.17
0100	65.00±2.43	-20.15	57.80±1.71	-28.99	53.80±3.00	-33.91	49.40±1.36	-39.31
1000	%MP	%MP	%MP	%MP	%MP	%MP	%MP	%MP

Conc., concentrations of the heavy metal in mg/ml; DFC, difference from control; MP, mortality of the crop; PF, pollen fertility; -, inhibition

Dharurkar (1971-Ph.D.Thesis) 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 in the present findings as well as by Salgare (2006).

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). However, Nair, Nambudiri and Thomas (1973) stated that it has

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

(Tested 3 weeks after initiation of flowering)
(Values given are mean ± SE of 500)
% Pollen fertility
Successive flowers

	F		F-24		F-48		F-72	
Conc.	%PF	%DFC	%PF	%DFC	%PF	%DFC	%PF	%DFC
0.001	19.00±2.36	+05.56	06.00±0.71	-28.57	4.40±0.87	-43.59	ng	ng
00.01	21.20±1.59	+17.78	05.20±0.86	-38.09	3.80±0.58	-51.28	ng	ng
000.1	12.00±1.05	-33.33	04.00±0.71	-52.38	2.80±0.86	-64.10	ng	ng
0001	09.00±2.21	-50.00	02.80±0.79	-66.67	1.00±0.55	-87.18	ng	ng
0010	06.00±1.30	-66.67	01.00±0.45	-88.09	ng	ng	ng	ng
0100	01.00±0.32	-94.44	ng	ng	ng	ng	ng	ng
1000	%MP	%MP	%MP	%MP	%MP	%MP	%MP	%MP

Conc., concentrations of the heavy metal in mg/ml; DFC, difference from control; MP, mortality of the crop; PF, pollen fertility; -, inhibition

Table 3 : Effect of Heavy Metal (copper sulphate - supplied through water) on the pollen tube growth of successive flowers of *Vigna unguiculata*

(Tested 3 weeks after initiation of flowering)
(Values given are mean ± SE of 500)
% Pollen fertility
Successive flowers

	F		F-24		F-48		F-72	
Conc.	%PF	%DFC	%PF	%DFC	%PF	%DFC	%PF	%DFC
0.001	224.00±15.66	-53.53	240.00±21.64	-52.19	106.00±17.46	-54.70	46.00±8.70	-55.77
00.01	204.00±15.34	-57.68	214.00±18.02	-57.37	098.00±19.81	-58.12	40.00±4.46	-61.54
000.1	184.00±11.64	-61.83	202.00±17.69	-59.76	086.00±11.21	-63.25	ng	ng
0001	150.00±20.21	-68.87	174.00±21.64	-65.34	070.00±09.98	-70.09	ng	ng
0010	128.00±10.18	-73.44	140.00±21.60	-72.11	060.00±08.93	-74.36	ng	ng
0100	nf	nf	nf	nf	nf	nf	nf	nf
1000	%MP	%MP	%MP	%MP	%MP	%MP	%MP	%MP

Conc., concentrations of the heavy metal in mg/ml; DFC, difference from control; MP, mortality of the crop; PF, pollen fertility; -, inhibition

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 extensive work of Salgare and Suwarna Gawde (2008) it could be concluded that the observations of Nair, Nambudiri and Thomas (1973) are superficial and misleading.

Inhibitation in the germination of pollen as well as tube growth was caused by the heavy metal of *V. unguiculate* (Tables 2 and 3). This proves that 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; Mhatre, Chaphekar, Ramani Rao, Patil, Haldar, 1980; Shetye, 1982 and Giridhar, 1984) as an indicator of pollution is a very crud method and rather a wrong choice. There is no evidence of any entire vascular plant exhibiting this much degree of sensitivity. Extensive work of Salgare and Suwarna Gawde (2008) also supports the present findings.

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