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MONITORING OF HERBICIDE (ATRATAF 50W) TOXICITY BY USING POLLEN AS INDICATORS - A CRITICAL REVIEW - I

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Potentiality of pollen germinability was noted in 10, 13, 11 series out of 20 series in each of the 5 species of the Apocynaceae[1-2], *Petunia grandiflora*[3] and *Petunia axillaris*[4] respectively. Atrataf 50 W suppressed the germinability of pollen in 2, 9, 5 series of the Apocynaceae, *Petunia grandiflora, Petunia axillaris* respectively. The herbicide stimulated the germination of pollen in 7, 2, o series of the Apocynaceae, *Petunia grandiflora, Petunia axillaris* respectively. Atrataf 50W stimulated the pollen tube growth in 5, 0, 0 series of the Apocynaceae, *Petunia grandiflora, Petunia axillaris* respectively. Inhibition in the germination of pollen was caused by atrataf 50 W in 7, 4, 5 series of the Apocynaceae, *Petunia grandiflora, Petunia axillaris* respectively. Herbicide inhibited the pollen tube growth in 7, 4, 5 series of the Apocynaceae, *Petunia grandiflora, Petunia axillaris* respectively.

Keywords : Atrataf 50w; Herbicides; Palynology; Toxicology.

The use of vegetation as biological indicator of environmental quality has a long history dating back to the miners canary, to the recognition about 100 years ago. Recent studies[5-8] have shown the feasibility of using natural vegetation for monitoring pollution.

Inspite of the very varied approach of study and the extensive work done, the larger number of herbicides being developed in industry and used in agriculture stand only in testimony of the necessity of more work in the field. Herbicides drastically reduced pollen germination as well as tube growth. It was, therefore, important to study the effect of such chemicals on germination as well as tube growth since inhibitory effects of these chemicals will eventually reduce fruit - and seed-set. For the present study Atrataf 50W very common herbicide was selected.

In recent years[1, 2, 4] the importance of gaining a knowledge on the germination potential of pollen in the bud stage of floral development has been realized, because of its possible applications in pollen storage and its subsequent use in plant breeding programmes. This is a new line of study and further work therein is likely to help in solving certain problems in hybridisation with this in mind the present work was undertaken.

Physiology of Pollen of Species Studied

To find out the germination potential of pollen in the bud stage of floral development, flower buds of various sizes marking the various stages of development and the open 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 soon after the dehiscence of the anthers (in open flowers) of the following plant species.

Salgare[1,2] studied 5 Cultivars of the Apocynaceae :

Nerium odorum Soland. Double-flowered (red-flowered cultivar)
Nerium odorum Soland. Single-flowered (pink- and white-flowered cultivars)
Vinca rosea L. (pink- and white-flowered cultivars) (recently named as *Catharanthus roseus* (L.) G. Don.

Sharma[3] studied 5 Cultivars of *Petunia grandiflora :*

Single-flowered : cascades (pink, red, white) cultivars
Double-flowered : duet and sonata cultivars

Salgare[4] studied 5 Cultivars of *Petunia axillaris* BSP. :

Light-violet-, pink-, violet-, white- and white-violet-flowered cultivars.

Methodology

Germination of pollen grains of successive flowers of the above plant species was studied by standing-drop technique in an optimum concentrations of sucrose [20% for F series of red-flowered N. *odorum*, both the cultivars of *V. rosea* and F-24 series of red-flowered *V. rosea*, 50% for F series of pink- and white- flowered *N. odorum,* F-24 series of both the cultivars of *V. rosea* and F-48 series of pink-flowered *V. rosea* and 80% for F-72 series of pink-flowered *V. rosea*[1,2]; 2% for F series of duet, F-24 series of white cascade and duet, F-48 series of white cascade, 10% for F series of pink cascade, sonata, F-24 series of pink and red cascades, sonata, F-48 series of pink and red cascades, 40% for F series of red and white cascades[3]; 10% for F-24 series of pink- and white- flowered, F-48 series of white-flowered, 30% for F series of white- and white-violet-flowered, F-24 series of light-violet- and white-violet-flowered, 50% for F series of light-violet, pink-, violet-floweres, 60% for F-24 series of violet-flowered cultivars of *P. axillaris*[4]] supplemented with a wide range of concentrations (10^{-17} - 10^{-2}- 10^{-3}, 1, 5, 10, 20-20-100 µg/ml) of atrataf 50W. Pollen grains were then transferred to a moist filter chamber, stored at room temperature (21-33°C) with RH 58 - 63% in the diffused laboratory light at the Institute of Science, Mumbai. The experiments were run in triplicate and average results were recorded. Observations were made 24 hours after incubation. For each experiment a random count of 500 grains was made (from different fields on the slide) to determine the percentage of pollen germination. For measurement of length of pollen tubes, 50 tubes were selected randomly and measured at a magnification of 100x. Percentage of inhibition (-) and stimulation (+) was also determined.

Results and Discussion

As a rule the percentage of pollen germination is always less than the pollen viability. However Dharurkar[9] (1971) reported higher percentage of pollen germination than the pollen viability in *Eichhornia crassipes*. With reinvestigation Salgare (1986c, 95) proved superficial and misleading observations of Dharurkar.

Salgare[1,2] recorded the potentiality of pollen germinability in F series of all the 5 cultivars of the Apocynaceae studied. It was the pollen of F-24 series of red-flowered cultivar of *Nerium odorum* and both the cultivars of *Vinca rosea* found germinated in the optimum concentrations of sucrose. It should be pointed out that the pollen of F-48 and F-72 series of pink-flowered cultivar of *Vinca rosea* showed their germination in the optimum concentrations of sucrose. Thus the potentiality of pollen geminability was observed in 10 out of 20 series investigated. Sharma[3] stated that the potentiality of pollen germinability in 5 cultivars of *Petunia grandiflora* studied was reported in 13 out of 20 series investigated. Pollen of F and F-24 series of all the 5 cultivars showed their germination in the optimum concentrations of sucrose. However, pollen of F-48 series of all the 3 cascades found germinated in the optimum concentrations of sucrose. Salgare[4] observed the potentiality of pollen germinability in F and F-24 series of all the 5 cultivars of *Petunia axillaris*. However, it was the pollen of F-48 series of white-flowered cultivar of *Petunia axillaris* showed their germination in the optimum concentration of sucrose. Thus the potentiality of pollen germinbility in 5 cultivars of *Petunia axillaris* was found

to be in 11 out of 20 series investigated. Johri and Chhaya Roy Chowdhury[10] stated that in *Citrulls colocynthis,* where pollen grains 'mostly remained attached in tetrads', satisfactory germination is observed.

Potentiality of pollen germinability was noted in 10, 13, 11 series out of 20 series in each of the 5 species of the Apocynaceae[1,2], *Petunia grandiflora*[3] and *Petunia axillaris*[4] respectively. Atrataf 50W suppressed the germinability of pollen in 2, 9, 5 series of the Apocynaceae, *Petunia grandiflora, Petunia axillaris* respectively. The herbicide stimulated the germination of pollen in 7, 2, 0 series of the Apocynaceae, *Petunia grandiflora, Petunia axillaris* respectively. Atrataf 50W stimulated the pollen tube growth in 5, 0, 0 series of the Apocynaceae, *Petunia grandiflora, Petunia axillaris* respectively. Inhibition in the germination of pollen was caused by atrataf 50W in 7, 4, 5 series of the Apocynaceae, *Petunia grandiflora, Petunia axillaris* respectively. Herbicide inhibited the pollen tube growth in 7,4, 5, series of the Apocynaceae, *Petunia grandiflora, Petunia axillaris* respectively.

Salgare[1,2] recorded the potentiality of pollen germinability even in F-72 series of pink-flowered cultivar of *Vinca rosea,* where he used the optimum concentration of sucrose as a culture medium. Sebastian[11], Sulbha Rane[12] andTrisa Palathingal[13] stated that the potentiality of pollen germinability was noted in F, F-24, F-48 series only in pink-flowered *Vinca rosea,* where they failed to germinate the pollen of F-72 series in Brewbaker and Kwack's Culture medium[14]. This proves that the culture medium is also have the bearing on pollen germination.

It should be pointed out that even the lowest concentration (10^{-17} µg/ml) of atrataf 50W tried suppressed the germinability of pollen of-24 series of red-flowered cultivar of *Nerium odorum* and F-72 series of pink-flowered cultivar of *Vinca rosea*[1,2], Sharma[3] stated that the germinability of pollen of F series of duet, sonata and F-24 series of pink, white cascades, duet and sonata and F-48 series of all the 3 cascades of the cultivars of *Petunia grandiflora* tried was prevented even by the lowest concentration (10^{-17} µg/ml) of atrataf 50W tried. Germinability of pollen of F series of white-flowered and F-24 series of light-violet-, pink-, violet-, white-flowered and F-48 series of white-flowered cultivars of *Petunia axillaris* was suppressed even by the lowest concentration (10^{-17} µg/ml) of atrataf 50W tried. This proves that the pollen of the said series are highly sensitive and acts as an ideal indicators of pollution.

The widest range of concentrations of atrataf 50W for the Apocynaceae was confirmed 10^{-17} - 100 µg/ml which stimulated the germination of pollen of F-24 series of white-flowered cultivar of *Vinca rosea*[1,2]. Sharma[3] recorded 10^{-17} - 10^{-15} µg/ml, the widest range of concentrations of atrataf 50W for *Petunia grandiflora* which stimulated the germination of pollen of F and F-24 series of red cascade cultivar. All the concentrations (10^{-17} - 100 µg/ml) of atrataf 50W tried to stimulate the germination of pollen of *Petunia axillaris*[4].

The widest range of concentrations of atrataf 50W for the Apocynaceae was confirmed 10^{-17} - 100 µg/ml, which stimulated the pollen tube growth of F-24 series white-flowered cultivar of *Vinca rosea*[1,2]. All the concentrations (10^{-17} - 100 µg/ml) of atrataf 50W tried to stimulate the pollen tube growth of *Petunia grandiflora*[3] and *Petunia axillaris*[4].

Sudhakaran[15] stated that in *Vinca rosea* besides pollen grains which produced

single pollen tube, it has also been noticed that tetraploid grains frequently produce more than one pollen tube. Pollen tubes are branched quite frequently. Aberrations of this type in the pollen tube development are not observed in diploid pollen tubes, but quite frequently met with the pollen grains of irradiated plants. Extensive work of Salgare[1,2] made it very clear that Sudhakaran had failed to trace out the branched pollen tubes and polysiphonous condition which is fairly common even in diploid pollen grains.

This proves that the herbicide (atrataf 50W) can be most successfully used as the growth substance which is very economical. This was already proved earlier by Salgare[1,2,4,16-24]. This was also proved by Salgare and Sharma[25-28], Salgare, Theresa Sebastian and Sharma[29-31], Sharma[3] and Singh[32]. Recently Salgare[33-34] again proved with the pollen of *Cyamopsis tetragonoloba* and *Phaseolus aureus* that the herbicides can be used most efficiently as growth substances.

The widest range of concentrations of atrataf 50W for the Apocynaceae was confirmed 10^{-17} - 100 µg/ml which inhibited the germination of pollen of F series of pink-flowered cultivars of *Nerium odorum* and *Vinca rosea*[1,2]. Sharma[3] reported 10^{-17} - 1 µg/ml, the widest range of concentrations of atrataf 50W for *Petunia grandiflora* which inhibited the germination of pollen of F series of pink cascade. Salgare[4] recorded 10^{-17} - 40 µg/ml, the widest range of concentrations of atrataf 50W for *Petunia axillaris* which inhibited the germination of pollen of F series of pink-flowered cultivar.

The widest range of concentrations of atrataf 50W for the Apocynaceae was confirmed 10^{-17} - 100 µg/ml which inhibited the pollen tube growth of F series of pink-flowered cultivars of *Nerium odorum* and *Vinca rosea*[1,2]. Sharma[3] reported 10^{-13} - 1 µg/ml, the widest range of concentrations of atrataf 50W for *Petunia grandiflora* which inhibited the pollen tube growth of F series of pink cascade. Salgare[4] recorded 10^{-17} - 40 µg/ml, the widest range of concentrations of atrataf 50W for *Petunia axillaris* which inhibited the pollen tube growth of F series of pink-flowered cultivar. From this it is confirmed that the pollen tube growth is more sensitive than pollen germination.

It should be pointed out that it is only Salgare[1-4] and his research group are dealing with the pollen physiology of successive flowers. With such an extensive work it is confirmed that pollen develoment and activity are more sensitive indicators of adverse factors in the botanical environment and the use of an entire vascular plant[5-8, 35-38] 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 was also proved earlier by the extensive work of Salgare[1-4, 17, 19, 23-31, 39-55].

The delay in pollen germination was interpreted by Saoji and Chitaley[56] as being due to the grains not being mature enough to effect pollination, immediately after being shed from the anther. Further they stated that 4-5 hours are required for the complete maturation of pollen grains. It was Salgare[1] for the first time proved that the pollen require resting period before gemination and it was the failure of Saoji and Chitaley who misinterpreted the resting period for pollen maturity.

Pollen germination and tube elongation are two distinct processes differing in their sensitivity to different concentrations of the herbicide was also confirmed with this extensive work. However, Nair *et. al.*[57] stated that it has been significant that the

optimum percentage of germination and tube length were attained in the same growth medium. With such a extensive work it could be concluded that the observations of Nair *et. al.* are superficial and misleading.

The weed control by chemical means has been replacing or supplementing traditional cultural methods in most of the agriculturally advanced countries. Due attention is being paid to the problem in these countries since the economic return from the crop is dependent upon it to a considerable extent. A large number of chemicals, inorganic, organic and auxin herbicides have been used for this purpose. Khosla[58] stated that no lethal doses could be determined for *Achyranthes aspera* and *Cassia tora* since maleic hydrazide recrystalised at room temperature beyond 5400 ppm. In fact it is apparent that the dosage required to kill the weeds through its conductive tissue is often sufficient to kill the crop as well.

Levi and Craft[59] and Molero and Blackhurst[60] have reported that MH does not show any residual effect on the soil. It should be pointed out that the findings of these workers are challenged by Salgare[48]. This shows that we must minimise the use of the higher concentrations of the herbicides.

Alternative method of weed control is suggested by Singhal and Sen[61] where they recommended that instead of killing the weed, suppress the seed germinability. It should be pointed out that their theory is challenged by Salgare[4] stating that Singhal and Sen have forgotten that it is the reproductive stage which consume maximum nutrients from the soil.

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