



ORIGINAL ARTICLE

**PGR ALTER MICRO AND MACRO ELEMENTS MORPHOLOGICALLY IN
GLORIOSA SUPERBA LINN.**

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ABSTRACT

In this study the five different treated plant samples of Glory lily were collected from field's of Udaiyarpalayam and analyzed for the possible presence of colchicines using Scanning Electron Microscope technique (SEM). The results of SEM have shown that the presence of elements, Ca and Fe are found more only in propiconazole treatment, also the quantitative estimation of Electron X-Ray Spectra observation confirms the percentage of Zn in gibberillic acid and *Pseudomonas aeruginosa* treatments were the highest among all the treatments and in control plants. In conclusion from the results, Glory Lily may be considered as colchicines sources for the chemical constituents of medicine industry. Further it would be useful of producing high amount of colchicines for pest control based on plant growth regulator and elicitor treatments.

Keywords: Colchicine, Scanning Electron Microscope (SEM), Electron X-Ray Spectra (EDS),

1.INTRODUCTION

One of the very important exported medicinal plants of India that has become endangered within a very short span of the last 50 years is *Gloriosa. Superba* L. (Family-Liliaceae). The root is used as a germicide, to cure ulcers, piles, haemorrhoids, inflammation, scrofula, leprosy, dyspepsia, worm's infestation, flatulence, intermittent fevers, and debility arthritis and against snake poison. (Wealth of India, 1948-76). The corm (or tuber) which looks like a hoe, It has been the most used in indigenous medical systems of India as well as in Africa. The medicinal importance of the plant is due to the presence of alkaloids (nearly 24 of them) of which colchicines and colchicoside are the principal ones, as well as to the presence of 10 non-alkaloid medicinal compounds including B-sitosterol, chelidonic acid, luteolin, stigmasterol etc (Nautiyal, 2011). The major alkaloids are colchicine, 3-demethyl colchicine and colchicoside Chitra and Kandhasamy). Seeds and tubers contain alkaloids such as colchicine and colchicoside, which are used to treat gout and

rheumatism (Trease and Evans, 1983). The colchicines content has been estimated to be 0.6% in seeds (Sarin et al., 1974) and colchicosides 0.8%. The seeds are the best source of colchicines as their content is 2–5 times higher than in tubers.

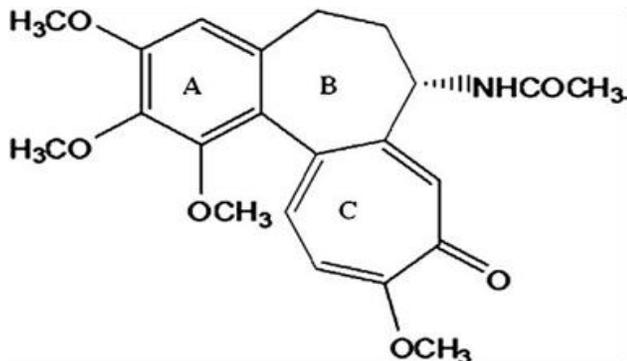
The medicinal importance of *G. superba* is due to the presence of alkaloids in all parts of the plant, mainly colchicine, an amino alkaloid derived from the amino acids phenylalanine and tyrosine (Sivakumar et al., 2004). The concentration of colchicine reported by Finnie and Van Staden, (1994) present in *G. superba* stem is 0.33–0.41%, in flower 1.18%, ovary 0.08%. Other compounds such as lumicolchicine, 3-demethyl-N-deformyl-N-deacetylcolchicine, 3-demethylcolchicine, and N-formyl-deacetylcolchicine have been isolated from the plant (Sugandhi, 2000; Suri et al., 2001). Various plant parts are used in spleen complaints, sores, tumors and syphilis. The extract of plant is CNS depressant (Kirthikar and Basu, 1935).

Colchicine is an alkaloid drug, chemically known as N-[(7S)-1, 2,3,10-tetramethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[a]heptalen-7-yl] acetamide, and widely used for the treatment of gout disease (Calogero, 1992). Colchicine has the high market value and consistent demand in the field of medicine (Bharathi et al., 2006). The alkaloids, colchicines is the drug of choice to relieve acute attack of gout and familial Mediterranean fever (Alali et al., 2004). At present there is renewed interest in the

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use of colchicines as a possible cure for cancer related diseases (Evans et al., 1981). Considering its significance this study will help in cultivation of *Gloriosa superba* for commercial extraction of colchicine.

Chemical structure of Colchicine



2.MATERIALS AND METHODS:

Medicinally important plant species, *Gloriosa superba* L. (Family: Liliaceae) was selected for the present investigation. The tubers were obtained from department of agriculture, Annamalai University, Tamil nadu, India. The triazole compound propiconozol was obtained from Syngenta, India Ltd., Mumbai. The plant growth regulator Gibberellic acid (GA₃) was purchased from Himedia India Ltd., Mumbai. The elicitor, *Pseudomonas fluorescens* was obtained from Krishi Care Bioinputs, Chennai, India. *Pseudomonas aeruginosa* was obtained from department of agriculture microbiology, Annamalai university, as culture form. During the study,

average temperature was 32/26°C (maximum/minimum) and relative humidity (RH) varied between 60-75 per cent. The experimental part of this work was carried out in Udaiyarpalayam (Ariyalur Dist) and Stress Physiology Lab, Department of Botany, Annamalai University, Tamil Nadu. The methodologies adopted are described below.

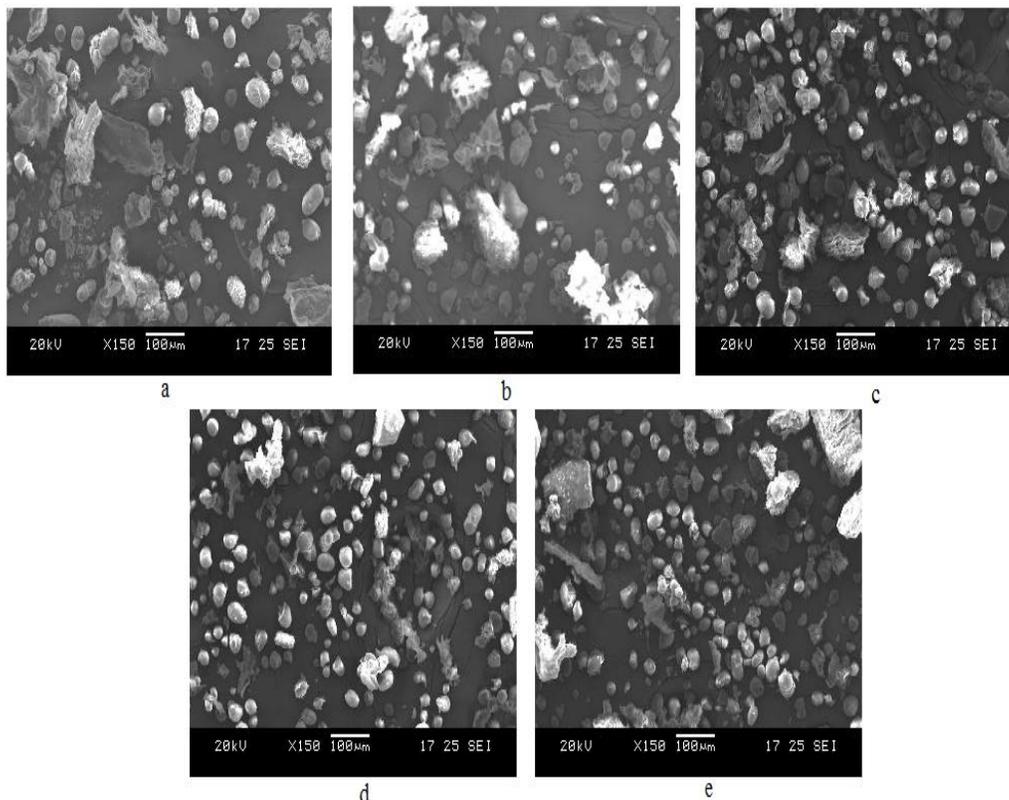
Cultivation methods

The plants were raised in field condition in Udaiyarpalayam (Ariyalur Dist) Tamilnadu India. The tubers were sown during 2010 (September) and 2011 (January) in a randomized block design with three replications. The experimental area was tilled and planting furrows (30 cm deep) made at a distance of 1.5 m, 20 days before planting. Potting mixture (red earth, sand, vermicompost and coir compost in1:1:1:1ratio) was applied in the furrows to ensure nutrient supply to the young plants. Each plot consisted of three 5 m long rows with inter and intra row spacing of 150 cm and 30 cm respectively. The plots were irrigated at weekly intervals. Recommended agronomic and plant protection practices were adopted.

Treatments

Five plots were selected by randomized block design (RBD). 10mg L-1 PPZ, 5 µm L-1 GA₃ and 1mg L-1 *P.fluorescens* and 1 slant of *P.aeruginosa* (mixed with 20g peptone, 1.5g MgSo₄, 1.5g K₂HPO₄, 10ml Glycerol with 1000 D.W), these concentrations were used for the treatment plants and control plants, irrigated with tap water. The treatments were given on 35, 50, 65 and 80 days after planting (DAS) by soil drenching and as spraying. The plants were taken randomly on 45, 60, 75

Figure 1: Morphological structure of plant of Glory Lily photographed at 20 kV under 1K magnification under (a) Control, (b) Gibberillic acid (c) Propiconazole (d) *Pseudomonas fluorescens*, (e) *Pseudomonas aeruginosa* treatments



and 90 DAP and separated into tuber, stem and leaves and used for determining non-enzymatic antioxidant contents.

Mineral content estimations

The samples were oven dried at 60°C for 1 hour. Then the whole plants were powdered well using an agate mortar. The powdered samples of Glory Lily were examined using Scanning Electron Microscope (JSM-5160), CISL, Annamalai University with an acceleration voltage of 20 kV. Before the observation, the samples were mounted on aluminium stumps and coated with gold in a sputtering device under vacuum. The presence of gold at the coated surface under study together with the accelerating voltage of the electron beam results in the production of excellent image of the material. Then the prepared samples are subjected to elemental analysis for the estimating of nutrients through SEM with EDS instrument.

3.RESULTS

Mineral content

Figure 1, show the elements present in the entire plant of *Gloriosa superba* under (a) Control (b) Gibberillic acid (c) Propiconazole (d) *Pseudomonas fluorescens* (e) *Pseudomonas aeruginosa* treatments. Figure 2, show the EDS reading of plant of *Gloriosa superba* (a) Control, (b) Gibberillic acid (c) Propiconazole (d) *Pseudomonas fluorescens*, (e) *Pseudomonas aeruginosa* treatments. Figure 3, show the (a) Macro and (b) Micro elements present in the plants of *Gloriosa superba* under the treatment and control plants.

4.DISCUSSION

In plants the elements K, Cu, Zn and Mg are commonly present in all the five treatments. Concentration of K, Cu, and Zn were found to be much higher in the treatments. Among the treatments, GA₃ showed less Mg contents than the other treatments and control. PPZ showed highest concentration of Na followed by GA₃, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa* and control. But in control plants the Na is present in very lesser concentration. Potassium is more in Gibberillic acid treatment when compare to PPZ. Even though potassium is not a constituent of important Organic compound in the cells, it is essential for the process of respiration and photosynthesis. PPZ showed highest concentration of Cu and Zn followed by GA₃ and control plants. Among the five treatments, PPZ shows the highest amount of Cu and Zn than the treatments and in control plants. Cu is necessary for nitrogen metabolism. It is bound tightly on Organic matter. Zn is involved in the functional part of enzymes including carbohydrate metabolism, auxin (growth hormones), protein synthesis and stem growth. Zinc deficiency may leads to iron deficiency (Fosmire,1990). The presence of Mineral and increase in mineral content due to organic manure treatment was noted in Glory lily (Ravi et al., 2011).

5. ACKNOWLEDGEMENT

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Figure 2: EDS reading of plant of Glory Lily (a) Control, (b) Gibberillic acid (c) Propiconazole (d) *Pseudomonas fluorescens*, (e) *Pseudomonas aeruginosa* treatments.

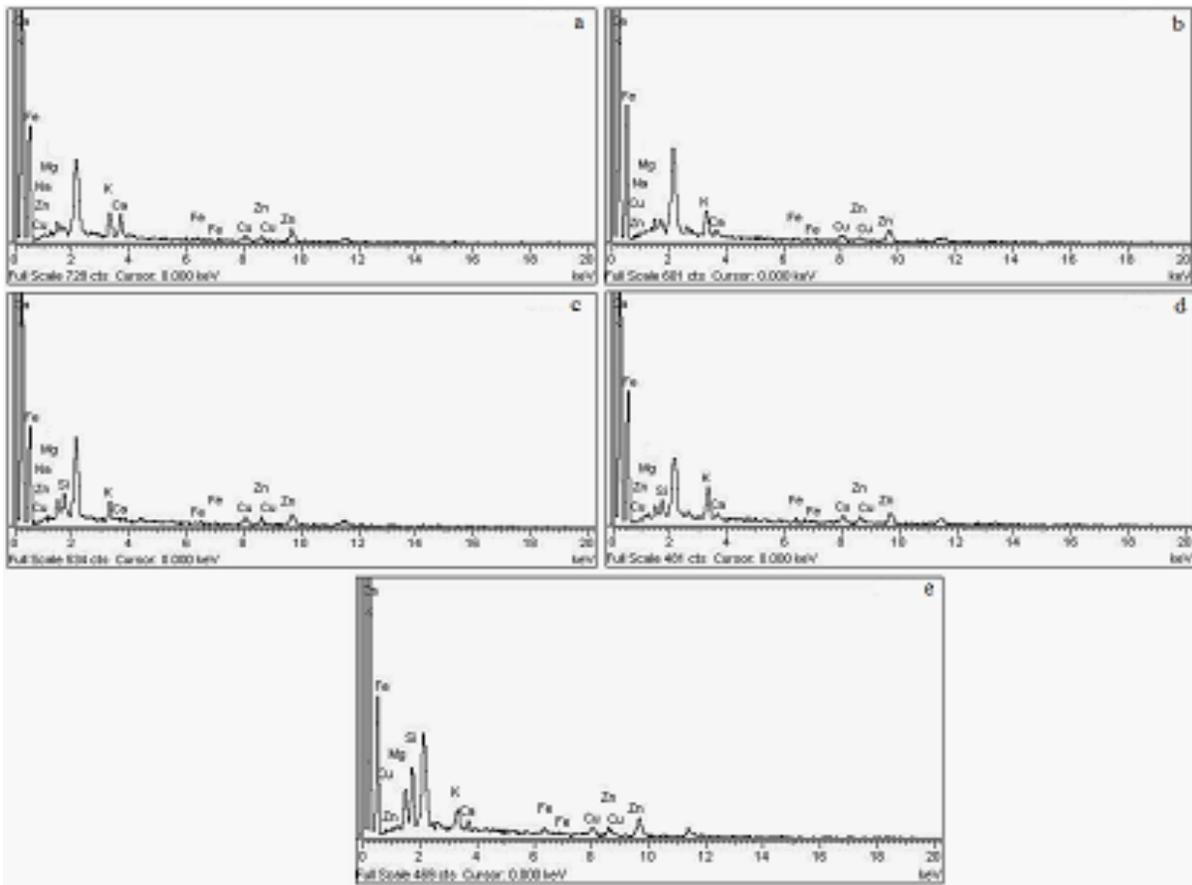
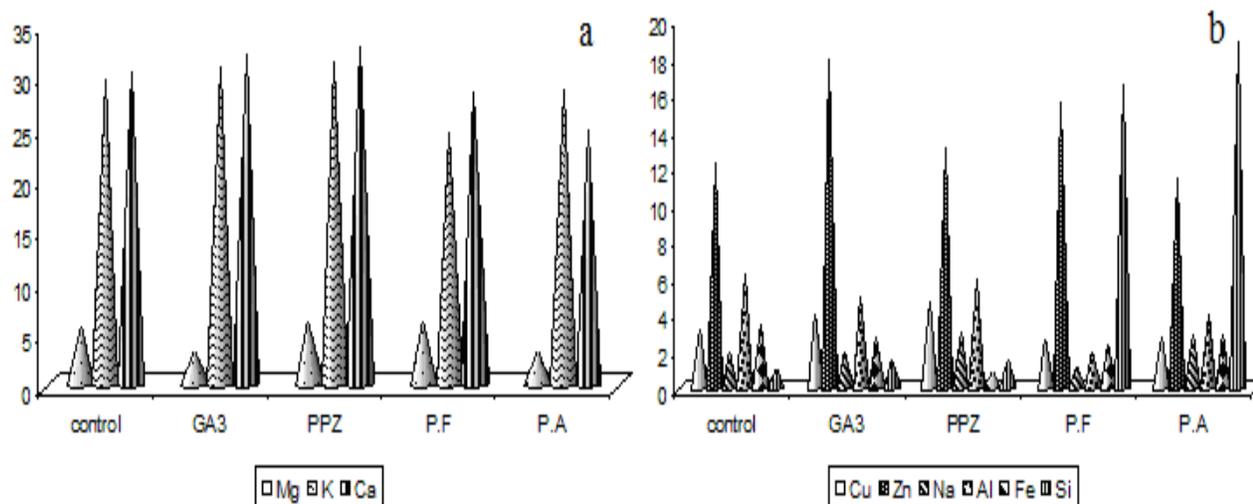


Figure 3: The (a) Macro and (b) Micro elements present in the plants of *Gloriosa superba* under the treatment and control plants.

6. REFERENCES

- Alali, F., Tawaha, K. and Qasaymch, R.H. 2004. Determination of Colchicines in *Colchicum steveni* and *C. hierosolymitanum* (colchicaceae): comparison between two analytical methods. *Photochem. Anal.*, 15:27-29.
- Bharathi, P., Philomina, D. and Chakkarvarthi, S. 2006. Antimitotic effect of colchicine from six different species of *Gloriosa superba* in onion roots (*Allium cepa*). *J.Med. Sci.*, 6 (3):420-425.
- Calogero, M. 1992. *Ortopedia e Traumatologia oggi* Anno XI (2) aprile.
- Chitra, R. and Rajamani, K. 2009. Genetic variability of *kazhappai kizhangu* (*Gloriosa superba* L.) in Tamil Nadu assessed using morphological and biochemical traits *Journal of Tropical Agriculture* 47 (1-2): 77-79.
- Evans, D.A., Tanis, S.P. and Hart, D.J. 1981. A convergent total synthesis of (\pm) colchicines and (\pm) Deacetoamidoisocolchicine. *J. Am. hem. soc.*, 103 (1981) 5813 – 5821.
- Finnie, J.F. and Van Staden. J. 1994. *Gloriosa superba* L. (flame lily): micropropagation and in vitro production of colchicines. In: Bajaj YPS, editor. *Biotechnology in Agriculture & Forestry*, vol. 26. Medicinal and Aromatic Plants VI. Berlin, Heidelberg: Springer Verlag; p.146–66.
- Plants VI. Berlin, Heidelberg: Springer Verlag; p.146–66.
- Fosmire, G.J. 1990. "Zinc toxicity" *Am.J.Clin.nutr.* 51 (2): 225-7.
- Kirithkar, K.R. and Basu, B.D. 1935. *Indian Medicinal Plants*. 2nd ed. Popular Publications Allahba, p. 2525–6.
- Kirithkar, K.R. and Basu, B.D. 1935. *Indian Medicinal Plants*. 2nd ed. Popular Publications Allahba, p. 2525–6.
- Nautiyal, O.P. 2011. Isolation of 3-demethylcolchicine from *Gloriosa superba* sludge and coupling with acetobromoglucose to yield colchicoside and thiocolchicoside. *Journal of Natural Products*, 4: 87-93.
- Ravi .S, Ashokkumar, S., Mallika, K. Kabilar, P., Paneerselvam, P. and Gayathri, M. 2011. Morphological, micro and macro nutrient analysis of the medicinal plant glory lily (*Gloriosa superba* L.) *Journal of Experimental Sciences* 2: 04-06.
- Sarin, Y.K., Jamwal, P.S., Gupta, R.K. and Atal, C.K. 1974. Colchicine from seeds of *G. superba*. *Curr Sci.* 43:87–90.
- Sugandhi, R. 2000. Biodiversity conservation and patenting and property right of tribal medicine of medicinal plants of India. 10th Asian Symposium on Medicinal Plants, Spices and other Natural products (ASOMPS X). Dhaka, Bangladesh, 18–23.
- Sugandhi, R. 2000. Biodiversity conservation and patenting and property right of tribal medicine of medicinal plants of India. 10th Asian Symposium on Medicinal Plants, Spices and other Natural products (ASOMPS X). Dhaka, Bangladesh, 18–23.
- Suri, O.P., Gupta, B.D, Suri, K.A. 2001. A new glycoside, 3-Odemethylcolchicine- 3-O-alpha-d-glucopyranoside from *Gloriosa* seeds. *Nat Prod Lett* 2001;15:217–9.
- Suri, O.P., Gupta, B.D, Suri, K.A. 2001. A new glycoside, 3-Odemethylcolchicine- 3-O-alpha-d-glucopyranoside from *Gloriosa* seeds. *Nat Prod Lett* 2001;15:217–9.
- Trease, S.E. and Evans, D. 1983. *Colchicum* seed and corm. In: *Pharmacognosy*, 12th edn. Balliere Tindall, London, pp. 593–597.
- vakumar, G., Krishnamurthy, K.V., Hahn, E.J. and Paek, K.Y. 2004. Enhanced in vitro production of colchicines in *Gloriosa superba* L. — an emerging industrial medicinal crop in south India. *J Horticult Sci Biotec*, 79(4):602–5.