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FORMULATION OF INCREASING ANTHOCYANIN CONTENT OF FLOWER OF *ROSA INDICA* L. PLANTS HAMPERED BY *BOTRYTIS* BLIGHT DISEASE BY 50% AQUEOUS-ETHANOLIC EXTRACT OF LEAVES OF *CALOTROPIS PROCERA* R.BR.

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ABSTRACT

Botrytis blight caused by *Botrytis cinerea* has hazardous effect on the red colour of garden rose (*Rosa indica* L.) flowers. The fungus *Botrytis cinerea* reduces the red colour of the rose flowers and thus causes a marked reduction of the market value of the rose flowers. The present work was undertaken to regulate the total anthocyanin content of *Botrytis* blighted horticulturally important rose plants that have been affected by *Botrytis cinerea* using active principles obtained from 50% aqueous ethanolic extract of leaves of *Calotropis procera*. Total monomeric anthocyanin concentration was measured here by the pH differentiation method, which is a rapid and simple spectrophotometric method. After partitioning the 50% aqueous- ethanolic leaf extract by using different solvents such as benzene, petroleum ether and chloroform, it was observed that the compound obtained from chloroform portion showed most inhibitory effect against *Botrytis cinerea*. Experimental findings indicated that *Botrytis* Blight reduced the total monomeric anthocyanin content. This reduction of anthocyanin content was recovered by using active principles obtained from chloroform extract of *Calotropis procera* and a remarkable increase of anthocyanin content was noted in the diseased plants.

KEYWORDS: *Calotropis procera, Botrytis cinerea,* spectrophotometric method.

INTRODUCTION

Botrytis blight, caused by the fungus *Botrytis cinerea*, is a very common disease of rose. Symptoms develop on all above-ground parts including flowers, buds, canes, and growing tips. Small, tan flecks or patches appear on flower petals and flower buds. Infected flower buds may droop and fail to open. The use of natural compounds of plant origin to protect crop, vegetables, fruits and flowers is an important means of developing biopesticides. *Calotropis procera* belongs to the family Asclepiadaceae, a giant milk weed, known for its anti-microbial properties for centuries.[1] Leaf extract of *Calotropis procera* has antifungal properties.[2] Antifungal effect of ethanol, aqueous and chloroform extracts of leaf and latex of *Calotropis procera* was studied against *Aspergillus niger*, *Aspergillus flavus* and *Microsporium boulardii*.[3] It is reported that the major floral pigments are flavonoids, carotenoids and betalains.[4] Anthocyanins are water-soluble phenolic compounds and present in different plants products such as vegetables, flowers and fruits, and are responsible for a wide range of colours from colourless to purple.[5] Final colour formation of flower depends on the structure, type and concentration of Anthocyanins.[6] Roses are very beautiful flowers that can form different attractive colour and for this reason they are used as ornamental flowers in different occasions. Anthocyanins along with others phytocompounds are primarily responsible for this wide range of colour formation in roses. *Botrytis* blight has a deleterious effect on rose flower. The present study was undertaken as a management protocol for the regulation of this deleterious effect of *Botrytis* blight disease in *Rosa indica* using active principles extracted from 50% aqueous ethanolic leaf extract of *Calotropis procera* in producing the red colour of the flowers.

MATERIALS AND METHODS

Plant material

Calotropis procera leaves were collected from Bamanpukur, Sree Mayapur, Nadia during the month of June –July 2013. The plant material was identified at the field using standard keys and descriptions.

Preparation of plant extract

Healthy *Calotropis procera* plant leaves were collected from Bamanpukur, Sree Mayapur, Nadia during the month of June –July 2013. 200 gm of the powdered plant material was soaked in 2L. of 50% aqueous ethanol for 5 days and then filtered. Residue was repeatedly soaked with 50% aqueous ethanol and filtered until the extract became colourless. Then the

extract was partitioned over benzene, petroleum ether and chloroform. Each partitioned sample obtained from benzene, petroleum ether and chloroform was evaporated under reduced pressure and each residue was dissolved in propylene glycol and tested for antifungal activity. Compound obtained from chloroform extract showed better inhibitory effect against *Botrytis cinerea*.[7] Antifungal activity was screened by agar cup method.[8-10] In this work different concentrations of the sample obtained from chloroform extract were applied in the field treatment. Griseofulvin was used as fungicide.

Field Experiment

Samples obtained from chloroform extract were chosen for the field treatment. Different concentrations of the samples were prepared. Two different sets were made one for healthy plants and another for infected plants. Samples were applied to the plants by foliar spray method. Five foliar sprays were given at the rate of single spray in the two consecutive days. The spray was done for 10 days. Flowers were collected and total anthocyanin content was measured.

Extraction of Anthocyanins

Two different pH buffer solutions were prepared (pH 1 and 4.5).[11] 2gms of each frozen flower samples were separately crushed in 20ml of each buffer solution with pestles and mortars. The crude extracts were filtered with Whatman No. 1 paper. The filtrates were collected and pH of the extract was maintained. Necessary dilution of the test samples were made by mixing one part of test solution with five parts of buffer solution. Absorbance of all the test samples diluted with pH 1.0 buffer and pH 4.5 buffers were determined at both 520nm and 700nm. All the test samples were read versus a blank cell filled with distilled water.

Total monomeric anthocyanins determination

Concentration of total monomeric anthocyanins of flower samples was measured by pH differentiation method.[11] Total anthocyanin content was calculated by using the following formula.

Anthocyanin pigment (cyanidin-3-glucoside equivalents, mg/L) = $\frac{A \times MW \times DF \times 10^3}{€ \times l}$

Where A= ($A_{520nm} - A_{700nm}$)pH 1.0 – ($A_{520nm} - A_{700nm}$)pH 4.5;

MW = (molecular weight) = 449.2g/mol for cyanidin-3-glucoside;

DF = Dilution factor;

L = path length in cm (1cm);

€ = 26900 molar extinction coefficient, in L × mol^{-1} × cm^{-1} for cyanidin-3-glucoside;

10^3 = factor for conversion from g to mg ;

Three replicates were prepared for each experimental set and total monomeric anthocyanin content was determined mg/L by statistical software SPSS 13.

RESULTS AND DISCUSSION

Table1: Study on total monomeric anthocyanin concentration in respect to healthy and infected set.

Treatment		Total monomeric anthocyanins mg/L.
Healthy	Mean Std. Dev.	61.98±0.17
Healthy + 30 mg/ml	Mean Std. Dev.	62.77±0.13
Healthy + 40 mg/ml	Mean Std. Dev.	134.02±0.38
Healthy + 50 mg/ml	Mean Std. Dev.	114.50±0.12
Infected	Mean Std. Dev.	4.21±0.05
Infected + 30 mg/ml	Mean Std. Dev.	56.07±0.16
Infected + 40 mg/ml	Mean Std. Dev.	132.87±0.25
Infected + 50 mg/ml	Mean Std. Dev.	84.41±0.09
Infected + Fungicide(Griseofulvin)	Mean Std. Dev.	80.70±0.37

All the values expressed in the table are statistically analyzed by ONE WAY ANOVA TEST in respect of healthy and infected set and found significantly different at 5% level ($p< 0.05$).

Results indicated that foliar application of the sample obtained from *Calotropis procera* improved anthocyanin content of the healthy as well as the *Botrytis* Blight infected *Rosa indica* plants.

From all the experimental sets it is clear that all the sample treated plants showed higher anthocyanin content. It was also observed that infected plant after treating with phytocompounds obtained from chloroform extract showed higher anthocyanin content as expressed (cyanidin-3-glucoside equivalents), mg/L. It was observed that Healthy + 40

mg/ml sample treated plants grew flowers that had more anthocyanin content (134.02±0.38 mg/L) than any other sets. By ANOVA we found that this variation of total monomeric anthocyanin content always crossed the 95% confidence level (significant at 0.05 level). It is 2.16 times more than healthy set and 31.83 times more than infected set. All the results clearly indicated that others sample treated plants grew flowers with more monomeric anthocyanin content in respect of healthy set which are also statistically significant.

It was also observed that infected plants after treating with 40mg/ml sample also flowered having significantly higher anthocyanins content in respect of infected plants. Infected plants showed very low concentration (4.21± 0.05 mg/L) whereas infected plants treated with 40 mg/ml sample produced more anthocyanin (132.87±0.25 mg/L) which is almost 31.56 times more than infected set.

It can be inferred that *Calotropis procera* can be cited as an efficient source of active principles that increases production of anthocyanin pigment content of *Rosa indica* flowers. The protocol is cost effective and non-toxic. This approach for increase of the red colour of rose flowers will definitely gear up the market value of the horticulturists and the florists.

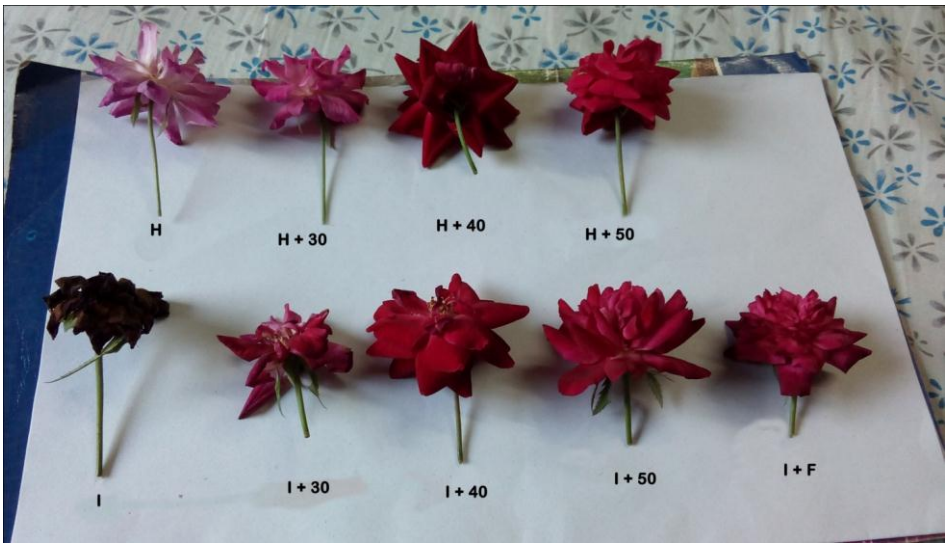
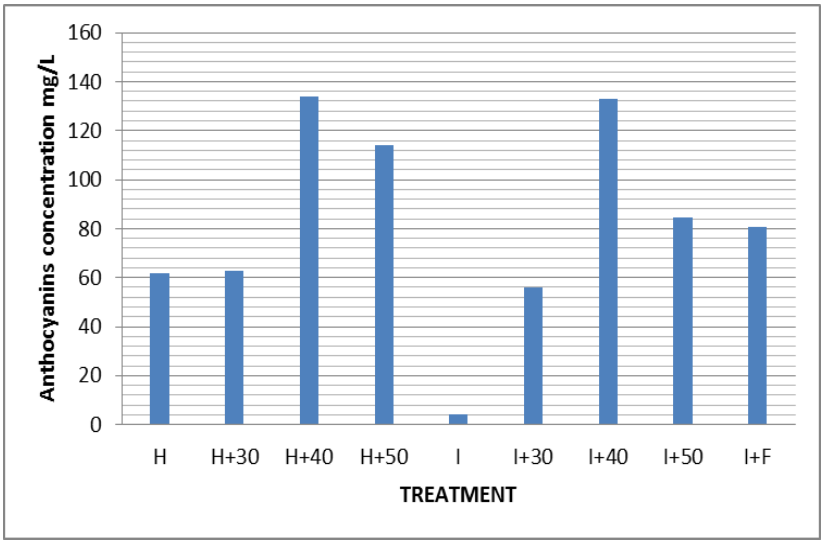


Fig 1: Colour variation of different flowers (H = Healthy, H+30= Healthy+ 30mg/ml sample, H+40= Healthy+ 40mg/ml sample, H+50= Healthy+ 50mg/ml sample, I = Infected set, I+30 = Infected + 30 mg/ ml sample, I+40 = Infected +40 mg/ ml sample, I+50 = Infected + 50 mg/ ml sample, I+F = Infected+Fungicide).



H= Healthy, H+30 = Healthy + 30mg/ml sample, H+40 = Healthy + 40mg/ml sample, H+50 = Healthy + 50mg/ml sample, I= Infected, I+30 = Infected + 30mg/ml sample, I+40 = Infected + 40mg/ml sample, I+50 = Infected + 50mg/ml sample, I+F= Infected+Fungicide treatment.

Fig 2: Graphical representation of all treatment sets showing variation in total monomeric Anthocyanin contents measured by pH differentiation method.

CONCLUSION

From the present study it is clear that healthy *Rosa indica* plants can produce more reddish flowers if active principles obtained from chloroform extract after partitioning 50% aqueous ethanolic leaf extract of *Calotropis procera* is used as foliar spray on regular basis. Thus it can be concluded that phytocompounds obtained from chloroform extract after partitioning 50% aqueous ethanolic leaf extract of *Calotropis procera* are promoter of monomeric anthocyanin synthesis. It can be also concluded that hazardous effect of *Botrytis* blight disease caused due to infection of *Botrytis cinerea* may be positively regulated by the use of phytocompounds obtained from chloroform extract after partitioning 50% aqueous ethanolic leaf extract of *Calotropis procera*. This management protocol for increasing red colour of rose flowers will be a highly beneficial and advantageous cost effective technique for florists and horticulturists for improvement of Rose flower quality.

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REFERENCES

1. Malik NN, Chughtai MID. Antimicrobial activity of *Calotropis procera*. A preliminary study. Pakistan J Sci, 1979; 31: 127-129.
2. Hassan SW, Bilbis FL, Ladan MJ, Umar RA, Dangoggo SM, Saidu Y. Evaluation of Antifungal Activity and Phytochemical Analysis of Leaves, Roots and Stem Barks Extracts of *Calotropis procera* (Asclepiadaceae). Pak J Biol Sci, 2006; 9(14): 2624-2629
3. Kareem SO, Akpan I, Ojo OP. Antimicrobial Activities of *Calotropis procera* on Selected Pathogenic Microorganisms. African J Biomed Res, 2008; 11(1): 105-110.
4. Tanaka, Y, Ohmiya, A. Seeing is believing: Engineering anthocyanin and carotenoid biosynthetic pathways. Curr. Opin. Biotechnol, 2008; 19: 190–197.
5. Del Valle, G. González, A, Báez R. "Antocianinas en Uva (Vitis vinifera) y su Relación con el Color," Revista de Fitotecnología, 2005; 28: 359-368.
6. Yoshida, K, Mori, M, Kondo, T. Blue flower color development by anthocyanins: From chemical structure to cell physiology. Nat. Prod. Rep, 2009; 26: 884–915.
7. Suraj S, Chatterjee P. Antifungal activity of 50% aqueous- ethanolic extract of leaves of *Calotropis procera* R.BR. International Journal of Scientific and Research Publications, 2015; 5(11): 230-235.
8. Perez C, Paul M, Bazerque P. Antibiotic assay by agar well diffusion method. Acta Bio. Med. Exp, 1990; 15: 113-115.
9. Alade PI, Irobi ON. Antibacterial activities of crude extracs of *Acalypha wilsiana*, J. Ethnopharmacol, 1993; 39: 171-174.
10. Abioye AO, Bamiro SB, Adesida SO, Hunpatin VP, Adeleke TI. Preliminary Phytochemical and Antimicrobial studies of *Phyllantus amarus* L. (Euphorbiaceae). Niger. Quart. J. Hosp. Med, 2004; 14: 282-287.
11. Lee J.; Barnes K.W.;Eisele T. Determination of Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, and Wines by the Ph differentiation method. Journal of AOAC International, 2005; 88: 1269 -1278.