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Biochemical changes in periwinkle (*Catharanthus roseus*) leaves infected with *Alternaria alternata*

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Abstract
Periwinkle (*Catharanthus roseus* L.), an important medicinal and ornamental plants growing worldwide. Periwinkle plant is attacked by *Alternaria alternata* (Fr.) Kessiler causing leaf blight disease. The disease was prevalent in all the periwinkle growing areas of Muzaffarnagar district, surveyed during 2018-19. Infection of periwinkle leaves with *Alternaria alternata* caused alternation in biochemical composition of leaves. Infected leaf as a result of 75% disease intensity exhibited significant reduction in total chlorophyll 1.42 mg/g, total sugar content 0.85mg/g, amino acid 0.62 mg/g and total alkaloids 1.87mg/g as compare to healthy leaves. The phenomenon was reversed in case of phenol content of leaf. Phenol content in healthy leave 1.25 mg was increased to 3.15 mg/g as a result of 75% disease intensity (table 1).

Keywords: Biochemical changes, periwinkle (*Catharanthus roseus*) *Alternaria alternata*

Introduction
Periwinkle (*Catharanthus roseus*) is a handsome garden plant and flowers throughout the year. This gives the plant the name “Sadabahar" (Jain, 1968) [9]. The whole plant has medicinal value (Janardhan, 2002) [10]. Leaves contain a higher percentage of VLB alkaloids like Vincristine and Vinblastine that are potent anticancer drugs (Aslam *et al.* 2010) [3]. Though; the periwinkle plant is hardy and particularly free from the attack of insect pests and diseases but is attacked by some insect pests and diseases. Some important diseases Which has been reported on periwinkle are the little leaf disease caused by phytoplasma, the dieback or twig blight or top rot caused by *Pythium butleri*, *Phytophthora nicotianae*, *Pythium debaryanum*, *Alternaria tenuissima* and Colletotrichum dematium. The other fungal disease reported on this crop are Fusarium wilt caused by *Fusarium solani* and *Sclerotium rolfsii*, blight caused by Myrothecium roridum, *A. tenuissima*, *A. alternata*, *Rhizoctonia solani*, *Ophiobolus catharnathicola* and *Glomerella cingulata*. In natural plantations of periwinkle, blight caused by *A. alternata* was observed as an important disease prevailing in and around Muzaffarnagar (U.P.). Observations of the plant under natural condition revealed that the Alternaria blight caused by *Alternaria alternata* (Fr.) Keissler was responsible for scattered spots followed by blighting of the leaves which ultimately results in severe defoliation of the plant. It is therefore, a direct loss of foliage of the plant that is of great medicinal value. Infestation of disease might also cause changes in the biochemical composition of leaves. Very little work has been done on biochemical changes in plants due to the infection of *A. alternata*. Present investigation was undertaken to see the effect of Alternaria blight on biochemical changes in periwinkle leaves.

Materials and methods
The pathogen *Alternaria alternata* was isolated, purified by single spore technique and maintained on PDA slants. Eight week old periwinkle plants were inoculated artificially by spraying the spore suspension having 1x 103 spores/ml with the help of an atomizer. The check was maintained by spraying the sterilized distilled water only. Treated and control plants were covered with polyethylene bags to provide identical moist condition for 48 hours and then exposed to natural conditions. Leaves showing different intensities of disease (compared with disease assessment key developed for this purpose) were collected for bio chemical analysis.

Estimation of chlorophyll
100mg of fresh leaves for each treatment (10,25,50,75% and healthy) were homogenised in 10 ml of 50% acetone. The homogenate was centrifuged at 5000 rpm for 10 minutes. The clear supernatant was transferred to a 25ml measuring cylinder. The residue was re–extracted with 5

ml of acetone centrifuged and supernatant was transferred to a measuring cylinde. The volume of supernatant was made up of 20 ml. using acetone. Absorbance of supernatant was determined at 663 nm and 645nm using 80 percent acetone as blank. The amount of chlorophyll a, chlorophyll b and total chlorophyll were calculated by using a formula proposed by Arnon (1956) [2].

Chlorophyll 'a' (mg/l) =12.7A663-2.69 A 645
Chlorophyll 'b' (mg/l) =22.9A645-4.68 A 663
Total Chlorophyll (mg/l) = 20.2A645 + 8.02 A 663
From these values chlorophyll (mg/gm) was calculated using following formula:

$$\text{Chlorophyll (mg/gm)} = \frac{\text{Chlorophyll (mg/l) X Total volume of extract (ml)}}{\text{1000 X sample weight (gm)}}$$

Estimation of total soluble sugars

Total soluble sugars were extracted in 80% ethanol, taking 100mg leaf sample for each treatment. The homogenate was centrifuged and supernatant was collected in clean tube. The pellet was re –extracted with 80 percent ethanol. Supernatant fraction thus separated was pooled and final volume was made to 10 ml with 80 percent ethanol. The extract was boiled and evaporated to dryness. The residue was dissolved in 4 ml distilled water and this extract was used for estimating total soluble sugars using anthrone reagent (Dubois *et al.*, 1956) [7].Aqueous sugar extract(0-5ml)was taken in clean test-tube and volume was made to 1.0 ml. with distilled water 5 ml anthrone reagent (2mg /ml in conc. H_2SO_4) was added slowly and mixed. Tubes were kept in boiling water bath for 10 minutes and then brought to room temperature. Absorbance of blue green colour was measured at 620nm.Glucose was used to prepare standard curve following the same method which has been used for calculating the soluble sugar content.

Estimation of Phenols

Phenols were extracted in 80% hot ethanol, taking 500mg of fresh leaves of each treatments (10,25,50,75% and healthy) following methods described by Swain an Hillis (1959) [21]. The homogenate was centrifuged and supernatant was collected in clean tube and final volume was made to 10ml with 80% ethanol. Aqueous Phenols extract (0.5ml) was taken in clean test-tube and volume was made to 1.0 ml with distilled water.0.5 ml Folion's reagent was added slowly in each tube and kept at room temperature for 3 minutes. 1.0 ml of 20% sodium carbonate solution was added, mixed and final volume made to 10 ml with distilled water. Tubes were kept in boiling water bath for one minute after cooling the test tube, absorbance was measured at 650 nm against blank. The phenols content was calculated using the standard curve prepared by taking tannic acid.

Estimation of amino acids

Total free amino acids in ethanol soluble fraction were estimated by ninhydrin reagent (Moore and stein,1948). In 0.5 ml aliquot, 0.5ml of distilled water was added followed by 1 ml ninhydrin reagent. samples were kept in boiling water bath for 20 minutes. After cooling, 5 ml diluents solution (n-propanol and water in 1:1 ratio) was added and absorbance was measured at 573 nm. Standard curve was prepared by taking known amount glycine. Ninhydrin reagent was prepared by dissolving 400 mg of ninhydrin and 16 mg Stannous chloride in a mixture of 10ml of 0.2M Citrate buffer (ph 5.0) and 10 ml of methyl cellosolve.

Estimation of total alkaloids

For estimation of total leaf alkaloids of Powered leaf (5gm) for each treatment was taken in a 250ml conical flask (Harborne, 2001) [8]. 100 ml. of solvent Mixture (69 ml eather +24 ml chloroform and 7.5 ml ethanol) was added to the flask, the flask was well- stoppered, shaken and allowed to stand for 10 minutes. Then 3 ml of NH4OH (10%) was added and the flask was shaken for one hour; and kept overnight.

Next day, 5 ml water was added to the flask. After shaking Vigorously, the contents of the flask were filtered through cotton in a separating funnel. Flask and cotton were further washed with a little ether and chloroform (1:1), and put in the separating funnel, contents of the separating funnels were extracted with N/2 H2SO4 (20+ 15+10 X3 ml) filtered and washed with water. Any residue was rejected.

Acid extract was made alkaline with ammonia solution + 5ml NH4OH (10%). Liberated alkaloids were extracted with chloroform (20+15+10x2ml) and chloroform layer is washed with water 10ml. Extracted with 2x5 ml of chloroform and filtered in the same flask chloroform was distilled until a few ml were left.

Alkaloids were then transferred into a preweighed phial (W1) gm.

Further alkaloids were dried to a final constant weight (W2) gm. The amount of total alkaloids were calculated by using the following formula.

Wt of alkaloids = (W2-W1) gm

$$\text{Alkaloid \%} = \frac{\text{(W2-W1) x 100}}{\text{Wt. of leaf powder}}$$

Result and discussion

Significant reduction in total chlorophyll and chlorophyll a and b was observed at all the levels of disease intensities under present investigation (Table). This may be due to the breakdown of photosynthetic apparatus because of infection of periwinkle leaves with A. alternata. The chlorophyll content reduced drastically when disease intensity increased from 10 to 50%.Total chlorophyll contents of leaves was minimum (1.42 mg/g) with 75 percent blight intensity followed by 50% (1.97mg/g) as compared to healthy leaves (3.60 mg/g). However, total chlorophyll content of chlorophyll a and b was the same at 10% and 25% disease intensity. The reduction in the photosynthetic pigment has also been reported in sunflower leaves infected with A. alternata (Kumar and Singh, 1996) [12] and in cluster beans infected with a Cucumerina var. yamopsis (Saharan and Saharan, 2004) [18] The reduction in chlorophyll content as the infection site may be due to various reasons like degradation of chloroplast (Mayer *et al.*, 1960) [13] destruction of chlorophyll due to infection (Diener,1963) and inhibition of its synthesis by the fungus (Pero and Main, 1970) [17].

Infection with *A. alternata* caused a significant reduction in total soluble sugar contents of periwinkle leaves. Sugar content of healthy leaves (3.10mg/g) was reduced to 2.87, 2.25, 1.45 and 0.85 mg/g in 10, 25, 50 and 75 percent blighted leaves respectively (Table-1).However, reduction in total soluble sugar content was quite low at low intensity of disease i.e. 10% and was at par to control at 5% level of significance. The reduction in sugar content may be correlated with the fact that the greater proportion of the plant nutrients is utilized by the pathogen (Vyas and Panwar, 1976 and Bhaskaran and Kandaswamy, 1977) [4].Partly it may also occur due to increased metabolic activities in infected in Alternaria disease

of sunflower (Kumar and Singh, 1996) [12] and cluster bean (Saharan and Saharan, 2004) [18].

Phenol content of periwinkle leaves increased significantly due to infection of *A. alternata* (Table-1).The highest Phenol content (3.15 mg/g) was observed in leaves having 75% disease intensity followed by 50% disease intensity (2.99mg/g).Similar result was observed by Sugha *et al*. (1992) [20] in onion downy mildew caused by Peronospora destructor. Kumar and Singh (1996) [12] have also reported significant increase in phenol content of sunflower leaves infected with Alternaria blight up to 40days of inoculations. Similarly, Joshi *et al*. (2004) [11] recorded an increase in total phenols of cluster bean leaves infected with *A. cucumerina* var. *cyamopsis*.

The total amino acid content of periwinkle leaves was significantly reduced to infection of *A. alternata* (Table-1) 10,25,50 and 75 per cent infection of leaves resulted in amino acid contents of 1.28,1.02,0.85 and 0.62 mg/g, respectively as compared to 1.75 mg/g in healthy leaves. Results of amino acids metabolism vary with host variety infected and the pathogen combinations. Naik and Addy (1973) [15] reported that there was no correlation between amino acids content and infection by *Cercospora personatum* in peanut. The amino acid proline disappeared in a rice plant infected with *Gibberella fujikuroi* (Andal and Subba Rao, 1956) [1]. Shekhawat and Kothari (1971) [19] investigated the amino acid composition of downy mildew infected plants of opium poppy and reported that concentrations of several amino acids were either reduced or remained unchanged in diseased leaves. Apple infected with *Aspergillus niger* showed reduction in asparagine, aspartic acid, threonine in Kesari Variety (BIsen, 1975) [5]. Thus nitrogen metabolism of fungal-infected host tissues appears to be very complex. Further studies with regard to decrease or increase in individual amino acid under pathogenesis of *A. alternata* may prove rewarding.

The total alkaloids contents of periwinkle leaves was significantly reduced due to infection of *A. alternata* (Table-1). 10%,25%,50% and 75%infection of leaves resulted in alkaloids contents of 3.38mg/g,2.90mg/g,2.38 mg/g and 1.87mg/respectively as against to 3.87mg/g in healthy leaves

Conclusion

Alternaria leaf blight is an economically important disease, prevalent in almost all periwinkle growing areas of Muzaffarnagar district and is a potential threat to periwinkle cultivation. Infection of periwinkle leaves with A. alternata caused alternation in biochemical composition of leaves. There was significant reduction in chlorophyll, total sugar, amino acid and alkaloids whereas, phenol content of leaves was increased due to infection.

References

1. Andal R, Subba Rao NS. Amino acid changes in the healthy and foot rot infected rice. J. Indian Bot. Soc. 1956; 35:414-416.
2. Arnon DJ. Chlorophyll absorption spectrum and quantitative determination. Biochem. Biophys. Acta. 1956; 20:449-461.
3. Aslam J Khan, Siddiqui SH, Fatima ZH, Maqsood Z, Bhat M, Nasim MA *et al*. *Catharanthus roseus* (L.) G (Don): An Important drug: It`s application and production. Pharmacie Global (IJCP). 2010; 4:1-16.
4. Bhaskaran R, Kandaswamy TK. Carbohydrate metabolisum of sunflower leaves infected by *Alternaria helianthi*. Phytopath medit. 1977; 16:103-108.
5. Bisen P. Changes in amino acid content of healthy and Aspergillus niger affected apple tissue. Indian Phytopath. 1975; 28:131-133.
6. Diener T. Pridicting and measuring transpiration based on phytomonitoring of tomato in green house. Ann. Rev. Phytopath. 1963; 1:197-218.
7. Dubois M, Gilles AK, Hamilton JK, Rebers PA, Smith F. Colourimeteric method for determination of sugars and related substances. Anal Chem. 1956; 28:350-356.
8. Harborne JB. Phytochemical methods. A guide to modern technique of plant analysis London, New York, 2001, 182-201.
9. Jain SK. Medicinal plants. National Book Trust, Green Park, New Delhi, India, 1968, 14-15.
10. Janardhanan KK. Diseases of major medicinal plants DAYA publishing house Delhi, 2002, 107-121.
11. Joshi UN, Gupta PP, Vivek Gupta, Sandeep Kumar. Biochemical factors in clusterbean that impact *Alternaria blight* resistance. J. Mycol. Pl. Pathhol. 2004; 34:581-583.
12. Kumar Rajiv, Singh SB. Influence of weather factors on Alternaria leaf spot development in sunflower. Indian J. Mycol Pl. Pathol. 1996; 26:234-236.
13. Mayer BS, Anderson DB, Bohning RH. Introduction to plant Physiology, Van Mostrand Company New York, 1960, 188p.
14. Morre S, Stein WH. Photometic method for use in chromatography of amino acids. J. Biol. Chem. 1948; 176:367-388.
15. Naik R, Addy SK. Free amino acid changes in peanut foliage in relation to Cercospora leaf spot diseases. Indian Phyto path. 1973; 26:245-248.
16. Neema DK. Changes in sugars of apple due to *Alternaria alternata* infection. Indian Phytopath. 1983; 36:626-629.
17. Pero R, Main C. Chlorosis of tobacco induced by alternariol monomethyl ether produced by *Alternaria tenuis*. Phytopath. 1970; 60:1570-1573.
18. Saharan MS, Saharan GS. Changes chlorophyll and non-strctural carbohydrates in clusterbean leaves infected with *Alternaria cucumerina* var. J. Mycol Pl Pathol. 2004; 34:500-504.
19. Shekhawat PS, Kothari KL. Amino acid compositon of healthy and downy mildew plants of opium poppy (*Papaver somniferum*). Indian Phytopath. 1971; 24:403-405.
20. Sugha SK, Develash RK, Singh BM. Biochemical alterations induced by Peronospora destructor in onion leaves. Indian Phytopath. 1992; 45:464-466.
21. Swain T, Hillis WF. The phenolic constituents of *Prunnis domestium*, the quantitative analysis of phenolic constituents. J. Sci. Food. Agric. 1959; 10:63-68.
22. Vyas NL, Panwar KS. Observation on nutritional changes in pomegranate fruit (*Punica granatum*) during pathogenesis by *Cladosporium oxysporum*. Comp. Physiol. Ecol. 1976; 1:97-99.