*Recent Research in Science and Technology 2011, 3(5): 57-61*
*ISSN: 2076-5061*
*www.recent-science.com*

PLANT PATHOLOGY

# BIOCHEMCIAL CHANGES IN POWERY MILDEW INFECTED LEAVES OF *ABELMOSCHUS ESCULENTUS*

V. P. Pawar[1]* and H. E. Rane[2]

[1]*Arts and Science College Bhalod, Tq Yawal, Dist. Jalgaon (M.S.), India*
[2]*Dhanaji Nana Mahavidyalaya Faizpur, Tq Yawal, Dist. Jalgaon (M.S.), India*

## Abstract

Four types of leaf samples $A_1$, $A_2$, $B_1$ and $B_2$ were taken from healthy and infected plants; and their free amino acids, sugars and organic acid contents were analyzed chromatographically. In case of free amino acids lysine, arginine, asparatic acid, glutamic acid, glycine and phenylatanine were found in all. There was no qualitative difference except for lysine, which gets totally consumed during the course of pathogenesis. Quantitatively was a general increase in amino acid content in B1 sample. In all three sugars were detected, they were fructose, sucrose and raffinose in all the four sample. There was a gradual decrease in the concentration of fructose and sucrose during the pathogenesis. While raffinose was maximum $A_1$ in sample. Organic acid detected in these samples were sebacic acid, adipic acid, succinic acid, maleic acid and citric acid. Succinic acid was totally missing in $B_2$ sample. While adipic and citric acid were maximum in $B_2$ sample. The maximum concentration of sebacic acid was $A_1$ sample and was least in $B_2$. The concentration of maleic acid was maximum in $A_2$ sample.

Keywords: *Abelmoschus esculentus*, *Erysiphe cichoracearum*, amino acid, sugar, organic acid

## Introduction

*Abelmoschus esculentus* or okara is one of the most important cultivated crop is widely distributed in India. This gets attacked by the powdery mildew fungus *Erysiphe cichoracearum.* After the initial stages of infection the fungus grows very rapidly, forms, oidial stages and covers the entire least surface within a short period so that it appears white mass powder. With decrease in temperature numerous black small cleistothecia are formed dispersed along with oidia imparting slightly grayish colour in the infected leaves.

However, there is possibility of some accumulatory damage to the crop due to metabolic disturbances caused by powdery mildew fungi. With this view in mind the present investigation was taken up to analyse the matabolites such as free amino acid, sugars and organic acid in healthy and infected leaves by chromatographic techniques.

## Materials and Methods

The following samples were collected from okara plant.

1. Young healthy leaf -A1
2. Old healthy leaf -A2
3. Young leaf just bearing oidial stage -B1
4. Old leaf mostly infected bearing oidia and cleistothecia -B2

(Referred to as perithecia in plates)

### Preparation of leaf extract

The leaf extract of all the samples was prepared using the method of Ranjan et al., (1955) and then the alcoholic extract was depigmented by healting with alumnia and carbon charocal (1 gm each / 10 ml).For thin layer chromatography (TLC) Silica gel G (NCL, Poona) was used Uni and two dimensional paper chromatography was used for the separation of amino acids, sugars and organic acids. In all the experiments Whatman No. 1 Chromatographic paper was used. The concentration of the spot of each sample extract was kept 0.05 ml using blood pipette. Different solvent systems and detecting reagents used were as follows:-

- Solvent system for amino acids – n–butanol, galcial acetic acid, water (60:20:20 V/V).
- Spraying reagent - 0.3% ninhydrin was prepared by dissolving it in 100 ml of acetone and 3 ml of glacial acetic acid.
- Solvent system for sugars – n– butanol, glacial acetic acids, water (90:10:25 V/V)
- Spraying reagent - 0.5 ml anisaldehyde + 9ml ethanol (90%) + 0.1 ml galcial acetic acid + 0.5 ml conc. $H_2SO_4$.
- Solvent system for organic acids - Ethanol ammonium hydroxide (25%) water (100:16:12 V/V)
- Spraying reagent – 0.04 gm of bromocresol green was added to 100 ml of ethanol and to this was added 0.1 N NaOH till a blue colour develop.

* Corresponding Author

In all cases the solvent run was given twice up to the same height. Sprayed chromatograms were developed at 105$^0$C for 10 minutes. All the chemical and reagents used were of analytical grade. The semi quantitative estimation of free amino acid, sugars and organic acids was done by taking the optical density of each spot form paper chromatograms by photo electric densitometer.

Fig. 1: Young healthy leaves of *A. esculents*

Fig. 2: Old healthy leaves of *A. esculents*

Fig. 3: Young infected leaves of *A.esculents*

Fig. 4: Old infected leaves of *A. esculents*

Fig. 5: Spores and perfect stage of *E. cichoracearum* occur on infected leaf *A. esculents*

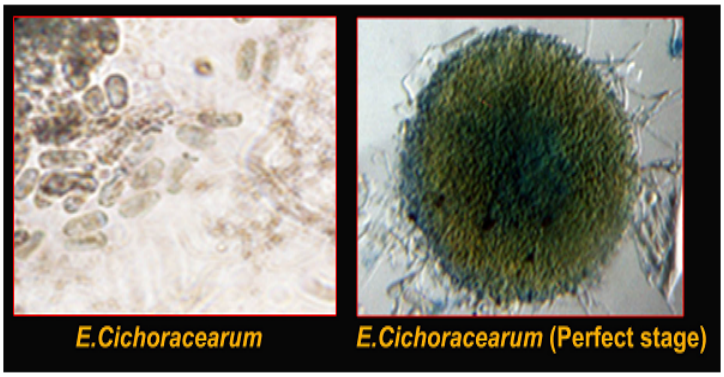


Fig. 6: Free Amino acid content of healthy and infected leaves *A. esculentus* caused *E. cichoracearum*

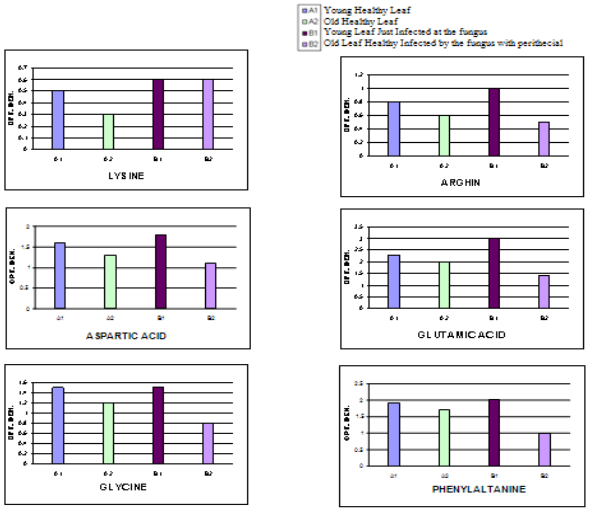



Fig.7: Sugar content of healthy and infected leaves *A. esculentus* caused *E. cichoracearum*

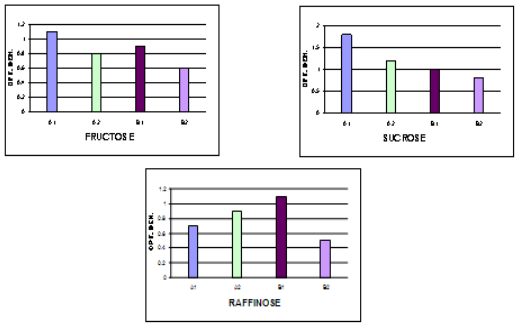
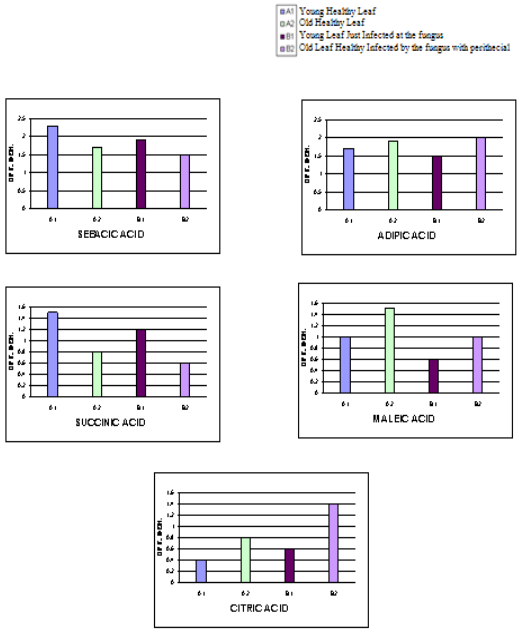



Fig. 8: Organic acid content of healthy and infected leaves *A. esculentus* caused *E. cichoracearum*



## Results

Figures 6 shows that in all 6 free amino acids could be detected from healthy and infected leaf extracts. No qualitative difference could be observed except for lysine, which was absent in $B_2$ sample; Quantitatively however, the concentration of each amino acid decreased in older healthy leaves. On infection, the concentration of each amino acid was found to be increased during early stages of infection, but in case of older and healthy infected leaves again all the amino acids were found to be reduced in their concentration.

The amount of fructose and sucrose was found to the less in old healthy leaf as well as in the infected young and old leaves. Raffinose was slightly increased in just infected young leaf, but again the concentration was to lowered in old mostly infected leaf extracts (Fig 7).

It was found that succinic acid was absent in $B_2$ sample. $A_1$ Sample shows maximum concentration of sebacic acid, adipic. Citric acid have been found to be in greater concentration in $B_2$, while maleic acid was maximum in $A_1$ sample (Fig 8).

## Discussion

*E. cichoracearum* is obligate parasite affect the physicological activities of the infected leaves of Okara. Most of the soluble organic substances of the host cell are readily utilized by the pathogen. A supply of this substance is regulated by the hydrolysis of the host macromolecules. As a result of hydrolysis or as a product of biosynthesis induced in pathogenesis, additional soluble organic substance would also appear. During the present institute it was found that the concentration of all amino acids found in greate young healthy leaves than in old healthy leaves, which might be due to the partial utilization of amino acids by mature leaves. Alternation in the metabolism in infected leaves has taken place in such a way that the concentration of each amino acid was increased during the early stages of infection, when the leaves were just covered by the mycelial and oidial stages of the pathogen, probably due to fact that at this stage both host and parasite were at their maximum metabolic activity, and causes more and more addition of amino acid to free amino acid pool. However, in advanced stages of inflection the concentration of amino acids starts decreasing which may indicate that the capacity to produce more amino acid was reduced or more utilization took place than their production. The same trend was found by Suryanarayana, *et al*. (1968) was studying die back affected cirtus trees. Several other workers e.g. Hanks and Feldman (1966); Andal and Subba Rao (1956); Howell and Krusberg (1966) also found similar trends in course of their studies on physiology of infected and healthy leaf.

Here lysine was found totatlly missing in old infected leaf indicating there by that lysine was not produced at all this stage and whatever was produced in initial stages was completely utilized by the host pathogen or in production of lysine is so slow that whatever was being produced was utilized, and nothing was left as free lysine in their leaf.

There was no qualitative difference in sugars in healthy and infected leaves but with regard to their quantitative difference it is clear that the concentration of fructose was greater in $A_1$ sample, while it was least in $B_2$. That it appears during pathogenesis the amount of fructose gets decreased. Also similar trend was noted for sucrose, which was also more in $A_1$ in comparsion to other sample and was least in $B_2$. Similar trend was found by Allen (1942) which suggested that reducing sugars in wheat leaves decreases due to inflection of powdery mildew powdery mildew. Dayal and Joshi (1968) also regard that the reducing, non-reducing and total sugar contents decrease in barely inflected with rust. The concentration of raffinose was greater in $B_1$ sample as compared to $A_1$ and $A_2$. It appears as initial stages of inflection have stimulated the formation of more raffinose, was utilized at this stages. However, the sugar also gets diluted at advanced stages of infection. The similar trend was found by Gerwitz and Durbin (1962) in rusted wheat leaves where there was subsequent decline during sporulation level below those in healthy leaves.

In all, five organic acids could be detected from all samples except for succinic acid, as it was absent in $B_2$ sample. It was found due to the alternation in the metabolism, which has taken in such a way that either the succinic acid was not produced in heavily infected leaves. The concentration of sebacic acid was found to be decreased following the infection, while adipic and citric acid concentration were increased in the diseased leaved particularly in $B_2$ sample. Maleic acid was also present in greater quantity in $B_2$ sample than $A_1$ and $B_1$ but the amount of this acid was maximum in $A_1$ sample.

## References

Allen, P. J. (1942). Changes in the metabolism of wheat leaver induced by infection with powdery mildew. Am. J. Bot., 29 : 425-435.

Andal, R., and Subbu Rao N. S. (1956). Amino acid changes in the healthy and foot infected rice (*Oryza sativa*). J. Indian bot. Soc., 34 : 414-416.

Dayal, R., and Joshi, M. M. (1968). Post infection changes in the sugar content of leaf spot infected barley. Indian Phytopath, 21 : 221-232.

Gerwitz, D. L., and Durbin, R. O. (1963). Some metabolic changes in wheat due to stem rust infection and different parasites. Phytopathology, 50 : 636.

Hanks, R. W. Feldman (1963). Composition of free amino acids and amides in the roots of healthy and *Redopholus simillis* infected grape fruit seedlings. Phytopathology. 53 : 419-422.

Howell, R. K., and L. R. Krusberg. (1966). Changes in the concentration of nitrogen, free and bound amino acid contents in Alfalfa and Pea infected by *Ditylenchus dipsaci*. Phytopathology. 56 : 1170-1178.

Rajan, S. Govindjee and M. M. Laloraya. (1955). Chromatographic studies on amino acid metabolism of healthy and diseased leaves of *Croton sparsiflorus*. Pree. nain Inst. Sci. India. 21 : 42-47.

Suryanarayan, D. R., Upadhya, and B. L. Chona (1968). Amino acids status of dieback affected Citrus in India. Indian Phytopath. 21 : 118-120.0.