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Biochemical changes caused due to *colletotrichum gleoesporides* in *Piper longum*

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Piper longum is one of the most important medicinal crops. Pimpri is widely used in ayurvedic and unani systems of medicine. The present investigation have been undertaken to investigate the biochemical changes occurred due to infection of *Colletotrichum gleoesporides*. Potato dextrose agar media was found to be support maximum growth of test isolate. Among the amino acid containing media SB agar was best. The study reveled that sugar, total phenol, chlorophyll and protein contain of disease part have been reduced due to infection of *Colletotrichum gleoesporides*.

Key words : *Piper longum, Colletotrichum gleoesporides.*

INTRODUCTION

PIPER longum is an under shrub in its natural habitat. In India pimpri is widely used in Ayurvedic and Unani system if medicine. The whole spike which consists of minute fruits embedded in a fleshy rachis is used as medicine. Green long piper is also used for pickling and for culinary purpose. It is used as medicine for respiratory tract in human being and also used in veterinary medicine.

The yield of dry spike during first year is occurred 400 kg/h to 1000 kg/ha fro third year the vine become less productive. (Vishwanathan 1995). The partial or total crop loss is found due to the infection of *Colletotrichum gleoesporides.* Piper longum is the host for *Colletotrichum gleoesporides* reported by Sathyarajan and Nassema, 1985.

The pathogen appears during the high humidity in atmosphere. The pathogen attacks the leaves and berries. Elliptical to oblong spots of variable six appear on both surfaces of leaves. In case in sever infection the loss may be more than 50% (Ramkrishna, 1954). As the piper longum being used as a medicinal the quality is entirely depend upon the biochemical constitutes, such as total sugar, phenol, protein, and oleoresin. The metabolic changes in the parasite have been reported by various workers. Also the apparently healthy tissues, surrounding the lesion are biochemical differentiate form healthy area.

Hence, the present study were undertaken with the objective to know the biochemical changes produced in the leaf , stem, and berry infected by *Colletotrichum gleoesporides* .

MATERIALS AND METHODS

Growth on different media

Five different solid media i.e. PDA and four amino acid containing media viz. SB agar, Elliott' agar, Brown's agar, and glucose isoleucine agar were tested.

For preparing amino acid containing media agar was dissolved in 500 ml water by heating. Remaining ingredient except amino acid was dissolved in 500 ml water. both the solution were mixed together and volume was made to 1000 ml. sporulation from each media was determined by taking 5 bit's of 5mm diameter. Then these were added in each 10 ml distilled water and shaken vigorously. The content was filtered through muslin cloth. The drop of homogenous suspension was taken on slide for counting. The observation were recorded by following the procedure

Abundant	+ + + +	more than 100
Good	+ + +	51 to 100
Moderate	+ +	26 to 50
Scanty	+	10 to 25
Nil		No suspension.

Biochemical Analysis

Estimation of total soluble sugar

The total soluble sugar has been estimated by following the method of Dubois et.al (1956). 100 ml sample was taken in boiling tube and 25 to 30 ml of ethanol was added and shaken over vertex mixer. After 20 to 30 min the settled material was filtered through Whatman's No.41 paper and again ethanol was added and repeated the procedure. Hot sand bath was used for evaporation of ethanol. 10 ml of water was added to sample and transfer to 100 ml flask. The volume was made to 100 ml by giving 2 to 3 washing with water. From this 1 ml aliquot of different sample was taken and 1 ml distilled water was taken as a blank tube. 1 ml of 5% phenol was added to tube and shaken vigorously and 5 ml of 96% H2SO4 was added and shaken vigorously on vertex mixer. The absorbance of golden yellow colour was read at 490 nm, against blank. The weight of total soluble sugar was determined by using the standard curve of glucose in mg/g of sample.

Estimation of reducing sugar

Reducing sugar was estimated by DNS method given by (Dubois et.al., 1956). 1 ml aliquot 2 ml DNS reagent was added, stirred and boiled for 8 to 10 min on water bath, after boiling 3 ml distilled water was added and stirred. Absorbance was read at 540 nm. The weight of reducing sugar (mg/g) was determined by using the standard curve of reducing sugar.

Estimation of total phenol

The procedure given by Bray and Thorpe (1954) was adopted to estimate the total; phenol. Plant extract was prepared by alcohol evaporation, after extraction with 80% ethanol; 2 ml was taken in test tube by adding 1 ml of Folin Ciocaltue followed by addition of 2 ml of Na2CO3 solution. After shaking the tube for 1 min the cooled solution was diluted to 25 ml by adding distilled water. The absorbance was measured at 650 nm. Using the standard curve, total phenol content was estimated as mg catechol equivalent to per gram of sample.

Estimation of Total chlorophyll

Fresh sample of different plant parts were weighed 1 gm was cut and homogenized with excess of acetone. The supernant was filtered through Whatman paper no 42. the volume of filtrate is made to 100 ml. the volume of 5 ml extract was made up to 50 ml with 80% acetone. Absorbance of the solution was read at 645 nm & 663 nm against the solvent.

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