*Asian J. of Bio Sci. (2006) Vol. **1** No. 2 : 114-116*

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

Md. Shamsher Alam and V. V. Deshmukh*
Department of Plant Pathology, Dr. Punjabrao Deshmukh Krishi Vidyapeeth, Akola - 444 004 (M.S.) India

(Accepted : June, 2006)

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% H_2SO_4 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 Na_2CO_3 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.

* Author for Correspondence

The total chlorophyll was calculated as follows

Mg total chlorophyll /g tissue = 20.2(A645) + 8.02(A663) *V/ 1000*W

Where:-

A – Absorbance of specific wavelength.

V – Final volume of chlorophyll extract in 80% acetone.

W – Fresh weight of tissue extracted.

RESULTS AND DISCUSSION

Taking into consideration the growth and sporulation, the most favorable media was PDA followed by SB agar, while glucose isoleucine agar and Elliott's agar media are unfavorable.

Table 1 : Showing five most favourable media.

Name of media	Average mean colony dia.mm	Growth character	Sporulation.
PDA	90	Good growth, concentric growth of mycelium, white pink mycelium, conidia in mass pink colour	+ + + +
Brown's agar	88	Good, smooth margin, mycelium white	_
Elliott's agar	64	Moderate growth, circular margin, mycelium white later grayish	+
SB agar	80	Grayish mycelium, concentric ring, colony grayish	+ + +
Glucose isoleucine media	89	Slow growth , irregular margin white mycelium	+ +

Among the five media tested, PDA was found to be most favorable for maximum colony diameter on 8th DAI, followed by Brown's medium, SB agar, & Elliott's agar. The maximum sporulation was also found on PDA. The favorable effect of PDAwas reported by Sarap (1989), Ekbote et.al (1997) and Gawande (2003). Among the amino acid media maximum growth shown by Browns agar and maximum sporulation in SB agar.

Biochemical Analysis

Ethyl alcohol extract of different samples of piper longum were used for colorimetric estimation of reducing and total soluble sugar by DNS method.

The result from table 2 reveled that reducing , non reducing sugar and total soluble sugar have decreased in diseased parts. The reducing sugar decreased upto 70.27% in halozone and 94.23% in necrotic zone.while total soluble sugar decreased by 69.42% in halo zone and 94.04% in necrotic zone.

Maximum decrease of these biochemical was recorded in necrotic zone of leaf. Reduction of sugar content in disease tissue due to *Colletotrichum gleoesporides* in betelvine has reported by Naik et.al.(1988) and due to *Colletotrichum capsici* in betelvine by Singh and Yadav (2002). The reduction of reducing sugar might be due to increased activity of oxidative enzymes of pentose phosphate pathway as explained by Kosuge (1978).

The changes in sugar in disease samples of piper longum have been attributed to the ability of profuse fungal multiplication .(Nema , 1989).

Table 2 : Effect on reducing sugar, total soluble sugar, and non reducing sugar due to *Colletotrichum gleoesporides*

Sample	Reducing sugar (mg/g)	% increased (+) or decreased (-) over healthy	Total sugar (mg/g)	% increased (+) or decreased (-) in healthy	Non reducing sugar (mg/g)	% increased (+) or decreased (-) in healthy
Leaf						
Healthy	14.23		30.25		16.02	
Halo zone	4.23	- 70.27	9.25	- 69042	5.02	- 68.66
Necrotic zone	0.82	- 94.23	1.80	-94.04	0.98	- 93.88
Stem						
Helathy	10.23		23.0		12.77	
Diseased	2.05	- 79.96	5.50	76.04	3.45	- 72.98
Berry						
Healty	15.02		36.0		20.98	
Diseased	6.8	- 54.72	14.60	- 59.44	7.8	- 62.82

Table 3 : Total phenol content of different part of piper longum.

Content	Leaf			Stem		Barry	
	Healthy	Halo zone	Necrotic zone	Healthy	Diseased	Healthy	Diseased
Total Phenol (mg/g)	14.39	6.32	2.86	7.89	1.82	16.28	5.67
% increase (+) or Decreased (-) over healthy	--	- 63.03	- 80.13	--	- 76.93	--	- 65.17

The similar result were obtained by Ekbote (1997) and Tind and Randhawa (1957).

Total phenol are considered to play an important role in host resistance, however phenol as such are not directly involved

in host resistance rather the compared derived from phenol act against pathogen.

Total phenol content was maximum in barriers (16.28 mg/g) and minimum in stem (7.89 mg/g) in healthy samples. The maximum reduction in necrotic zone of leaves (- 80.13%)and minimum reduction has been found in halo zone of leaves (-63.03%).

However,content of total phenol is in accordance with the results of Naik et.al.(1988) . similarly in Khatri et.al. (1997) also found reduction in total phenol in leaf spot disease of betelvine.

Chlorophyll content

Total chlorophyll content (mg/g) was estimated using fresh samples of piper lingum by following procedure given by Subbarao et.al. (1979) and Shridhar et.al. (1976).

From table 3 decreased in total chlorophyll content over healthy samples was maximum in necrotic zone of diseased leaves and stem (- 99.35% followed by diseased barriers (-96.08%) and was maximum in the halozone of disease leaves (-52.40%).

Decreased in total chlorophyll content was recorded in all the disease samples and was maximum in necrotic zone of leaf. The reduction of chlorophyll content might be due to their reduction or inhibition of chloroplast development and stimulation of enzyme chlorophyllase which attack chlorophyll. The reduction of chlorophyll in plant due to infection by pathogen is supported by many workers. Subramanyam et.al.(1976), Borah et.al.(1978), Tofazzal Hussain (1999).

REFERENCES

Bray,H.G and W.V.Thorpe (1954). Estimation of total phenols from plant tissues .*Meth. Biochem. Annal*. **1:** 27-52

Borha,R.C.;K.L.Bajaj and I.S.Bhatia (1978). Biochemical changes in tea leaves after infection with red rust. *Phytopahthol.Z.* ***89 :*** *167-180*.

Dubois,M.; K.S.Gilles and F.Smith (1956). Colorimetric methods for determination of sugars and related substances. *Annl.Chem.* **28:** *350-356*

Ekbote,S.D.; G.N.Padayanur;M.S.Patil and S.N.Chattanwar (1997). studies on the cultural, nutritional aspects of *Colletotri chum gleoesporides,* the casual organism of mango anthracnose. *J.Mycol,Pl.Pathol* **27(2):***229-230*

Kasuge,T.(1978). The capture and use of energy of disease plant. In plant disease .*An.advanced Treaties Horsefall.J.G.and E.B. Eds.* **3:** *86-113.*

Khatri,R.K.;R.L.Keshwal and M.L.Nayak (1997). Biochemical changes induced by *Xanthomonas campe- stris* pv.beticola causing leaf spot of betelvine. *Pl .Dis .Res* .**12(1) :** *25-27.*

Naik,M.K.; P.C. Hiremath and S.V. Hiremath (1998). Post-infectional changes in the betel vine leaves infected with *Colletotric hum gleoesporides*. *Indian Phytop athology* **41(3):***370-372.*

Ramakrishna,T.S. (1954). Leaf spot of turmeric caused by *Colletotrichum capsici.*(syd)Butler and Bisby. *Indian Phytopathology.* **7:** *111-117.*

Sridhar,R;P.R.Reddy and A. Anjaneyalu (1976). Laboratory manual of biochemistry. Edited by Thimayya, publication UAS, Dharwad.1986.

Subrahamanyam,P;P.Ramgopal;M.Malakondiah and M.N.Reddy (1976). Physiological changes in rust infected groundnut leaves. *Phytopathol.Z.:***87:** *107-113.*

Subbarao, B. C; A. Ghosh and V.T.John.(1979).Laboratory manual of biochemistry. Edited by Thimayya, publication UAS, Dharwad.1986.

Sathyarajan,P.K and A.Naseema (1985). Piper longum – a new host record for *Colletotrichum gleoesporides*. *Current science 54 (13 – 637).*

Sarap,K.A (1989). Studies on leaf blight disease of sensevieria trifasciate caused by *Colletotri chum gleoesporides*. M.Sc. Thesis (unpub.) Dr.P.D.K.V. Akola.

Singh,S.B.and B.P.Yadav (2002). Changes in phenol and free amino acid content in betelvine leaves infected with *Colletotrichum capsici. RAU .J.Res.PUSA :* **12(1-2):** *11 -14.*

Thind,K.S and H.S.Randhawa (1957). Studies on nutrition of *Colletotri chum capsici* . the incidents of die back of chilli. *Current Science. : 26-17*

Tofazzaltossain, M. M. Zahangir and M.Asgar (1999). Changes in different nutrients and enzymes content in mango leaves infected with *Colletotrichum gleoesporides. Indian Phytopath* .**52(1):***75 – 78*