INTERNATIONAL JOURNAL OF PLANT PROTECTION | VOLUME 7 | ISSUE 1 | APRIL, 2014 | 125-127
e ISSN-0976-6855 | *www.researchjournal.co.in* | Open Access

IJPP

RESEARCH ARTICLE

Studies on toxin and enzymatic effect on *Choanephora infundibulifera* in soybean crop

■ KUNJLATA VERMA

Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya, RAIPUR (C.G.) INDIA

ARITCLE INFO

Received : 09.11.2013
Revised : 26.02.2014
Accepted : 11.03.2014

Key Words :
Choanephora infundibulifera, CMC, Pectine, Macerating enzymes, Vegetable pith

***Correspondance author:**

ABSTRACT

Maximum degradation of CMC (90.17 %) and pectin (91.56 %) was found at 4.0 and 7.0 pH, respectively by the culture filtrate of *Choanephora infundibulifera* and enzymatic activity up to 420 and 840 minutes in the reaction mixture of culture filtrate. The 100 per cent pure concentration of culture filtrate was most effective for macerating of potato, carrot and pumpkin pith.

How to view point the article : Verma, Kunjlata (2014). Studies on toxin and enzymatic effect on *Choanephora infundibulifera* in soybean crop. *Internat. J. Plant Protec.*, **7**(1) : 125-127.

INTRODUCTION

Choanephora leaf blight is the most serious disease of soybean causing heavy losses of crop throughout the world. Because of the importance of this fungus, to know the toxin and enzymatic effect of test pathogen on vegetable pith in lab conditions the experiment was undertaken at Indira Gandhi Krishi Vishwavidyalay Raipur, during 2009-2010.

MATERIAL AND METHODS

Determination of enzymatic activity :

Cellulase (Cx) activity :

Cellulase activity was determined by measuring the reduction in viscosity of 0.5 per cent carboxy methyl cellulose (CMC) solution (Muse *et al*., 1972). Viscometric measurements were made with Ostwald's viscometer at time intervals 0, 60, 120, 180, 240, 300, 360, 420 and 480 minutes. The reaction mixture consisted of the following :

- 5 ml of 0.5 per cent carboxy methyl cellulose solution.
- 2 ml of sodium citrate buffer (at pH level of 4, 7, and 9).
- 2 ml of enzymes preparation (Broth fungus culture filtrate).

The enzyme preparation heated to 100^0C for 10 minutes served as control. 10 days old culture filtrate solution was used in the experiment.

Preparation of culture filtrate :

Potato dextrose broth was used as medium. After autoclaving, the medium was inoculated with 7 mm disc of the test fungus (seven days old culture). When culture of the pathogen covered the upper layer of medium, culture filtrate was filtered with the help of funnel and Watsman filter paper and filtrate was used for enzymatic study.

Polygalacturonase (PG) activity :

Polygalacturonase activity was determined by measuring loss in viscosity of 1.2 per cent pectin solution (Muse *et al*. 1972). Viscometric measurements were made with Ostwald's viscometer at different time intervals *i.e.* 0, 60, 120, 180, 240, 300, 360, 420, 480, 540, 600, 660, 720, 780, 840, 900, 960 and 1020 minutes. The reaction mixture consisted of the following :

- 5 ml of 1.2 per cent pectin solutions.
- 2 ml of sodium citrate buffer (at pH level of 4, 7, and 9).
- 2 ml of enzymes preparation (Broth fungus culture filtrate).

For estimation of cellulase and PG activity, 10 day old culture filtrate solution was taken for the reduction in viscosity for CMC as well as PG was calculated by the following formula:

$$\% \text{ loss in viscosity} = \frac{T_0 - T_1}{T_0 - T_w} \times 100$$

where,

T_0 = flow time of reaction mixture at '0' minute.

T_1 = flow time of reaction mixture at a particular time interval.

Tw = flow time of distilled water.

Macerating enzymes :

Seven mm diameter and 1 mm thick disc of potato, carrot and pumpkin were prepared and sterilized with $HgCl_2$ (1: 1000) followed by three subsequent washings with sterilized water. Five concentrations of culture filtrate *viz.*, pure culture filtrate (100 %), 80 per cent, 60 per cent, 40 per cent, 20 per cent were used for estimation of macerating enzymes. Twenty ml of each concentration was taken in 90 mm sterilized Petri plates. Three discs were dipped in each concentration, disc dipped in sterilized water served as control. These Petri plates were kept at room temperature. Three replications for each concentration were maintained and observation of rotting of potato, carrot, and pumpkin disc was taken at every one hr interval.

RESULTS AND DISCUSSION

The data on the influence of culture filtrate of *C. infundibulifera* on the activity of CMC at different pH levels are given in Table 1, which clearly show that cellulolytic activity differed with the change of pH levels from 4 to 9 and time interval from 0 to 480 minutes. From the data, it is evident that per cent loss in viscosity was very high (90.17) at pH 4.0 as compared to 88.54 and 80.16 per cent at pH 7.0 and 9.0,

Table 1 : Influence of culture filtrate of *Choanephora infundibulifera* on the activity of carboxymethyl cellulase (CMC) at different pH levels

Time interval (in minutes)	Per cent loss in viscosity at different pH levels		
	pH 4.0	pH 7.0	pH 9.0
0	0	0	0
60	70.16	55.19	49.65
120	70.42	66.69	70.94
180	80.56	70.14	78.03
240	81.86	75.89	78.17
300	87.05	78.19	78.74
360	87.57	85.09	79.88
420	90.17	88.54	80.16
480	90.17	88.54	80.16

Table 2 : Influence of culture filtrate of *Choanephora infundibulifera* on the activity of polygalacturonase (PG) at different pH levels

Time interval (in minutes)	Per cent loss in viscosity		
	10 days old culture filtrate		
	pH 4	pH 7	pH 9
0	0	0	0
60	38.82	23.89	17.1
120	50.67	24.16	24
180	53.96	27.52	26.29
240	67.78	38.93	45.15
300	68.43	57.06	48.23
360	69.09	59.07	49.35
420	72.38	60.4	53.15
480	74.36	76.53	55.32
540	74.38	77.87	57.04
600	76.73	79.48	58.18
660	79.62	80.88	61.72
720	79.75	84.58	73.15
780	79.75	91.29	77.73
840		91.56	79.44
900		91.56	82.30
960			84.01
1020			84.01

Table 3 : Effect of culture filtrate of *Choanephora infundibulifera* on macerating of potato, carrot and pumpkin pith.

Concentration of culture filtrate (%)	Rotting of vegetable pith		
	Potato	Carrot	Pumpkin
20	61 hrs.	96 hrs.	93 hrs.
40	42 hrs.	88 hrs.	71 hrs.
60	36 hrs.	40 hrs.	48 hrs.
80	22 hrs.	36 hrs.	44 hrs.
100 (Pure culture filtrate)	18 hrs.	20 hrs.	23 hrs.
Control	105 hrs.	120 hrs.	144 hrs.

respectively. The per cent loss of viscosity was maximum at 420 minutes at all ranges of pH. The enzymatic activity in culture filtrate was started at 60 minutes and became maximum at 420 minutes. It is evident from the data that culture filtrate adjusted at pH 4.0 was most suitable for rapid degradation of CMC by the enzymatic activity of *C. infundibulifera*. The data on the influence of culture filtrate of *C. infundibulifera* on the activity of PG at different pH levels are given in Table 2 which clearly show that pectin activity differed with the change of pH levels.

From the data (Table 2) it was evident that the per cent loss in viscosity was very high (91.56 %) at pH 7.0 as compared to 84.01 and 79.75 per cent at pH 9.0 and pH, 4.0, respectively. The per cent loss of viscosity was maximum at 840 minutes at pH 7.0 followed by pH 9.0 and pH 4.0 at 960 and 720 minutes, respectively. The PG enzymatic activity started at 60 minutes and became maximum at 840 minutes. It is evident from the data that culture filtrate adjusted at pH 7.0 was most suitable for rapid degradation of pectin by the enzymatic activity of *C. infundibulifera*.

Lucas and Sherwood (1966) recorded polygalacturonase, pectin esterase and cellulase (Cx) from fungus *Alternaria alternata* when culture on modified Richard's solution with cellulose, starch as carbon source was tested. They suggested that polygalacturonide hydrolyses terminal linkages more readily than interior linkages of polygalacturonase chain. On a pectin salt's medium, the isolates produce polygalacturonase, pectinesterase and pectinlyase. The pectin lyase was most active on pectin at pH 7.2.

Umana and Ikotum (2000) also recorded enzymes exo-polygalacturonase, endo-polygalacturonase and endo-pectate lysate from *Choanephora cucurbitarum*. The endo-polygalacturonase was the predominant enzyme at a lower pH of 4.0-6.0, with the highest activity at pH 5.0.

The data presented in Table 3, indicate that the culture filtrate of *C. infundibulifera* at 100 per cent concentration was completely macerated in all vegetable pith of potato, carrot and pumpkin at 18 hrs., 20 hrs., 23 hrs., respectively. While 105 hrs. 120 hrs. and 144 hrs. were taken by control, respectively. The data showed the result 100 per cent pure concentration of culture filtrate was found maximum in quantity of both conductive and inductive for some hydrolic enzymes for penetrating the cutin layer and in 20 per cent concentration of culture filtrate, was found minimum quantity of these enzymes so penetration of cutin layer took maximum time as compared to higher concentration in all treatments used *i.e.* potato, carrot and pumpkin.

In a similar work done by Kapat *et al*. (1998), it was observed that liquid culture filtrate of *B.cinerea* produced both conductive and inductive forms of hydrolic enzymes and also suggested that enzymes may have important role in penetrating the cutin layer.

Binjhare (2002), observed that liquid culture filtrate of *B.cinerea* produced both conductive and inductive for some hydrolytic enzymes, which may have important role in penetrating the cutin layer. High concentration of culture filtrate of *B.cinerea* was reported to cause extensive cell death in potato tissue.

REFERENCES

Binjhare, L.P. (2002). Studies on grey rot (*Botrytis ricini* Godfrey) of castor (*Ricin communis* L.) M.Sc. Thesis, Department of Plant Pathology, I.G.A.U. Raipur (C.G.) pp. 10-11.

Kapat, A., Zimand, G. and Elad, Y. (1998). Biosynthesis of pathogenicity hydrolytic enzymes by *Botrytis cinerea* during infection of bean leaves *in vitro*. *Mycological Res*., **102**(8) : 1017-1024.

Lucas, G.B. and Sherwood, R.T. (1966). Role of pectic enzymes of *Alternaria tenuis* in brown spot disease of tobacco. Proceedings of the IVth International Tobacco Scientific Congress, Athens, 410, 416.

Muse, R.R., Couch, H.B., Moore, L.D. and Muse, B.D. (1972). Pectolytic and cellulolytic enzymes associated with *Helminthosporium* leafspot on Kentuckey blue grass. *Can. J. Microbiol.*, **18** (7) : 1091-1098.

Umana, E.J. and Ikotum, T. (2000). Effect of pH on enzyme productivity by three pathotypes of *Choanephora cucurbitarum*. *Global J. Pure & Appl. Sci*., **6**(3) : 413-418.