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Extraction of toxin of *Helminthosporium sativum* and its role in characterization of resistance in foliar blight of wheat

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Abstract
Pathogenic microorganisms release toxic substances which are related to post superficially toxin seems to play an important role in host recognition at the site of initial contact of germinating spores on host surface thus, enabling the pathogen in establishing symptoms. In this study TLC purified toxin, crude toxin and spore suspension were taken of all five virulent strain of *Helminthosporium sativum* on susceptible variety UP 2329 and see how they produce symptoms in laboratory. The lowest Rf value of all the isolates of *H. sativum* were found more toxic and helpful in symptom production along with its characterization. Toxin play important role to screen out a number of varieties within a short time which are susceptible or resistant against disease.

Keywords: Extraction, toxin, *Helminthosporium sativum*, characterization

Introduction
Cereal shows a pivotal role to satisfy world food demand increasing population particularly in developing countries where cereal-based production system is the only predominant source of nutrition and calory intake reported by Nikos and Jelle 2012 and Shiferaw *et al.* 2013 [9]. Globally wheat is cultivated in an area around 220 million ha. with record production of 763.06 million tonnes of grains. Maximum area under wheat is in India 14 per cent and production is 98.51 million tonnes. *Helminthosporium sativum* (*Bipolaris sorokiniana*) causing foliar blight is one of the most important foliar pathogens of wheat causing 2.7 to 100% losses in various countries depending on varieties (Villareal *et al.* 1995 and Singh and Srivastava 1997) [11, 10]. *Helminthosporium* is a serious pathogen of wheat in warm and humid region of the world such as south east Asia, America, China and Africa (Joshi *et al.* 2000). In leaf blight disease, toxin play an important role in etiology. There are some earlier reports on phytotoxic production which are sesquiterpenes and non- host specific (Apoga *et al.* 2002 and Kumar *et al.* 2002) [1, 7].

Materials and Methods
Many plant pathogens including *Helminthosporium* app. are known to produce toxins that contribute decisively in Production of disease symptoms. Some pathogenic isolates of *Helminthosporium sativum* (*Bipolaris sorokiniana*) were grown in liquid culture and partially purified toxin from culture filtrates were obtained by precipitation and extraction with chloroform and ethyl acetate. The liquid was evaporated by flash evaporator and the residues dissolved in absolute alcohol to get crude toxin. Purification of toxin was done through TLC eluting it in ethanol. In present study, spore suspension, crude toxin and lowest fraction of TLC purified toxin of different isolates of *Helminthosporium sativum* (H1, H2, H3, H4 and H5 isolated from susceptible variety UP2338 and HD 2329) were taken to find out the role of toxin in synonyms production and It's characterization.

Result and Discussion
Application of a drop of spore suspension of pathogen, crude toxin and TLC purified toxin and distilled water(check) separately on detached wheat leaves in *in vitro* condition, showed different size of lesion produced by different isolates. It was revealed from photograph and table (Table 1) that toxin preparation having lower Rf values were toxic in case of all isolates. It has already been demonstrated (Singh,1999) that lower fraction of toxin preparation from *Helminthosporium sativum* obtained by separation on TLC is comparatively more toxic than with higher Rf. values therefore, the toxin fraction with lowest Rf. values only were used.

The TLC purified toxin of isolate H5 was more toxic and produced larger necrotic areas and this was followed by H3, H4 and H2. The isolate H1 was least effective in producing foliar blight symptoms as showed by plate A.

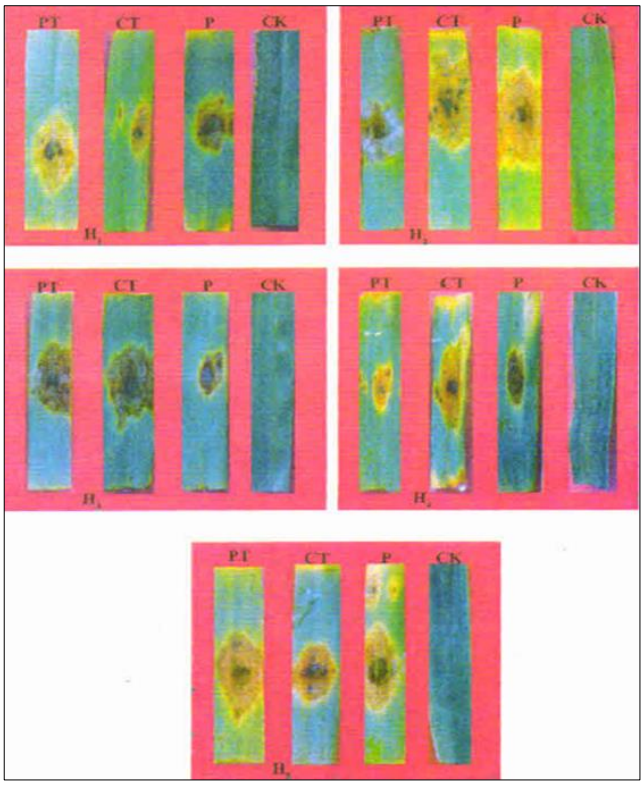
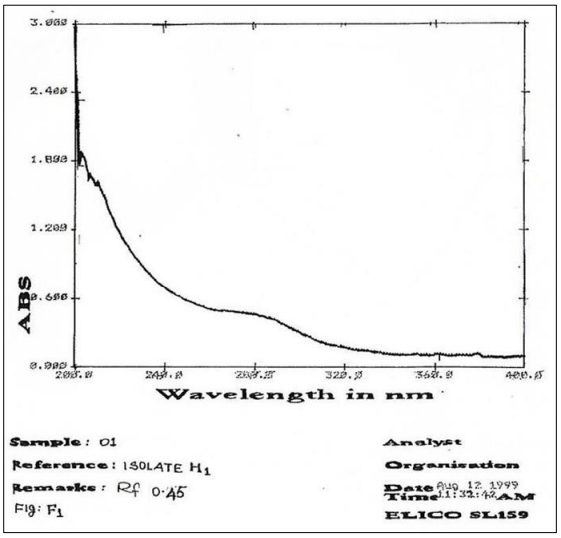


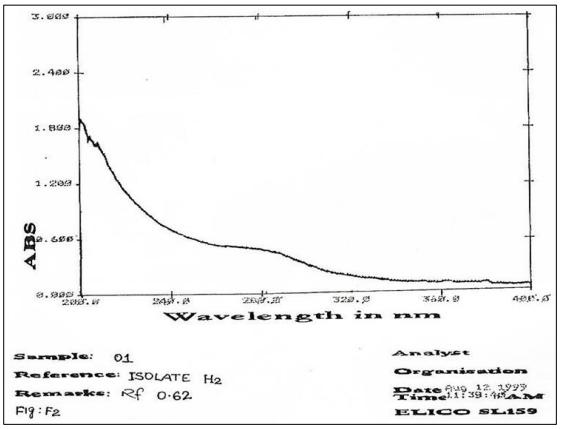
Plate A: Effect of toxin on symptom production

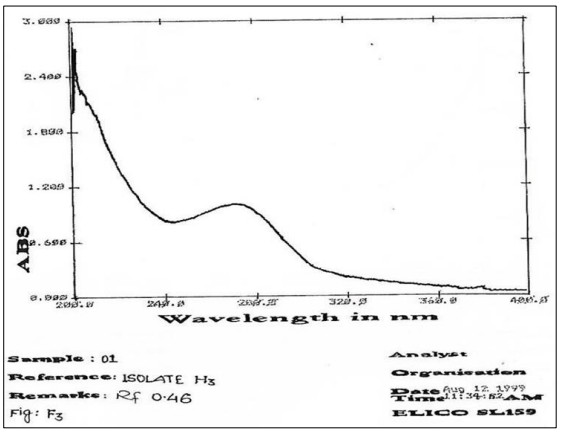
PT-Purified Toxin, CT- Crude Toxin, P- Pathogen, CK-Check

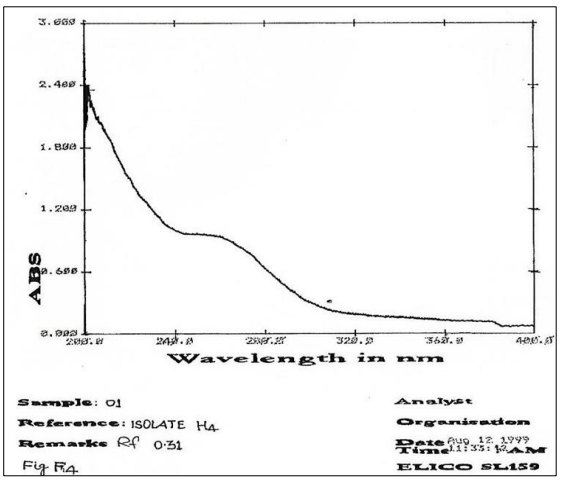
Characterization of toxin

Active principles isolated by preparatory TLC if crude toxin on silica gel using benzene: methanol (95:5) solvent system revealed more than one band in crude to inform each of the isolates suggesting the presence of more than one compound. Since the lowest fraction was most toxic in symptom production, therefore, the lowest fraction exhibiting fluorescence under UV light were marked and eluted in double distilled sterile water and pass through whatman no.42 filter paper to remove gel to be used as purified toxin for its characterization. The selected fraction of purified toxin of each isolate was scanned by UV visual spectrophotometer at UV wavelength of 200- 400nm to get absorption spectra. In case of isolates H1, the fraction had peak at 270nm wavelength with concentration of 0.45×10^{-4} moles/ litre. Isolates H2, H3, H4 and H5 had peak at 270, 275, 260 and 267nm with concentration of 2.045×10^{-4}, 0.68×10^{-4},2.045×10^{-4} and 2.0×10^{-4}moles/ litre, respectively. The toxin showed maximum absorbance at wavelength between 260-275nm indicating that the active principle in the fraction is Helminthosporal which has also been reported by Ghislaine and Closet (1978) and Wu *et al.* 1989 to have maximum absorbance between 260-290 nm. Gupta *et al.* (2006) [3] also isolated antifungal metabolites from bacteria. In the present study, we report new compound by different strain of *Helminthosprium sativum* that is "helminthosporal" which has been purified and characterized through TLC having wave length between 260- 270nm.the same work is also done by Jahani *et al.* (2006 and 2014) [4, 5].









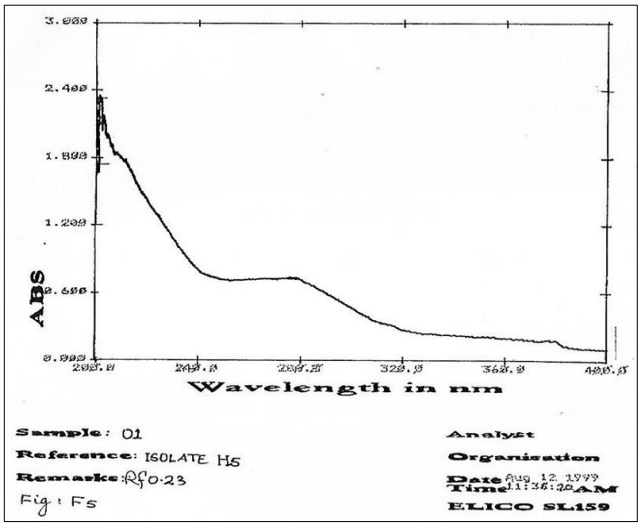


Table 1: Optical density and concentration of toxic metabolite of different fraction of *Helminthosporium sativum*

Isolates	R_f Values	Wave Length (nm)	OD	Concentration Moles/litre
H_1	0.45	270	0.56	0.45×10^{-4}
H_2	0.62	270	2.25	2.045×10^{-4}
H_3	0.46	275	0.75	0.681×10^{-4}
H_4	0.31	260	2.25	2.045×10^{-4}
H_5	0.23	267	2.20	2.0×10^{-4}

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