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ALTERNARIA LEAF BLIGHT OF WHEAT : PHYSIOLOGICAL STUDIES OF THE PATHOGEN AND ITS CONTROL

BY

A. K. BERA AND ANITA SAHA

Department of Biology, Basic Science & Humanities,
Bidhan Chandra Krishi Viswavidyalaya, Mohanpur,
Nadia, West Bengal, India

The present investigation has been undertaken to study the physiology of *Alternaria triticina* Pasad & Prabhu, causing leaf blight of wheat and method of controling disease. Best growth of *A. triticina* was observed in basal medium supplemented with cellobiose. Utilization of different carbon sources occured of a low rate in the early stage but rapid uptake of nutrients occured in the late stage when a bulk of mycelium was produced in the medium. Maximum utilization of nitrogen sources by the pathogen was observed when peptone was used as sole source of nitrogen. Analysis of the alchol extract of mycelial mat indicated presence of 13 amino acids, 4 sugars and 2 organic acids. Out of six fungicides tested (viz. captan, blitox, dithane M-45, dithane Z-78, vitavax and bisdithane) effective control of the pathogen both in vitro and in vivo was obtained with vitavax.

INTRODUCTION

Leaf blight of wheat caused by *Alternaria triticina* Prasad & Prabhu is reported from different States of India (Garg *et al* 1972; Kumar and Arya, 1973). Depending on the cultivated varieties of wheat, the disease sometimes appear so severe, reducing 20-80% yield of the crop. The disease is seed borne. Seed dressing with different fungicides fail to control the pathogen completely, although thiram and dithane M-45 showed 50% control at 0.5% concentration (Kumar and Arya, 1973). In the present investigation, physiological studies of the pathogen i.e. nutritional uptake, amino acid, sugar and organic acid content of *A. triticina* have been made. In vitro and in vivo test with different fungicides have been conducted to study evaluation of fungicides against leaf blight disease of wheat caused by *A. triticina.*

MATERIALS AND METHODS

Nutritional uptake was studied using basal medium supplemented with various carbon and nitrogen sources. The basal medium consisted of 0.1% KH_2PO_4, 0.05% $MgSO_4$, $7H_2O$, 2 ppm Zn^{++}, Mn^{++}, and Fe^{+++}. Erlenmeyer flask (250 ml) containing sterile liquid medium was inoculated with 7 days old mycelial mat (5 mm diameter) and incubated at 30°C. At the end of incubation period mycelia were collected, repeatedly washed with distilled water to remove all traces of liquid medium, dried at 60°C for 96 hours and weighed.

For identification of free amino acid, sugar and organic acid, the fungus was grown on Czapek-Dox liquid medium (pH 6.0) at 30°C for 15 days. Mycelial mats were then extracted with 80% ethanol, centrifuged at 200,000 rpm for 15 minutes. The supernatant was evaported to dryness in a rotary vacuum evaporator and dissolved in 80% ethanol for analysis.

Amino acids were detected by two dimentional TLC method using n-butanol : acetic acid : water (4 : 1 : 5 v/v) as first solvent and water saturated phenol as second one. The dried chromatogram was sprayed with 0.2% ninhydrin in n-butanol and heated at 60°C for 10 minutes. For sugar, water saturated phenol was used as the first solvent and ethylene : pyridine : water (8 : 2 : 1 v/v) as the second one. The dried chromatogram was sprayed with anilinepthalate reagent and heated at 105°C for 10 minutes. For organic acid, n-butanol : acetic acid : water (15 : 3 : 2 v/v) was used as the solvent system. The dried chromatogram was sprayed with 0.04% bromophenol blue in 95% ethanol. Suitable reference standards were used for identification of different components.

Six fungicides tested (viz. captan, blitox, dithane M-45, dithane Z-78, vitavax and bisdithane) were incorporated seperately into warm potato-dextrose-agar (PDA) medium, so as to reach 50, 100, 500, 1000 and 2000 parts per million (ppm) concentration respectively. The mixed media were shaken and poured in 9 cm diameter sterilized petriplates. The plates were inoculated with 7 days old mycelial mat (5 mm diameter). Control plates in which no fungicides were added, inoculated similarly. Four replicates were made in each treatment. The plates were incubated at 30°C for 10 days after which diameter of growth was recorded and LD_{50} values of different fungicides were calculated.

Evaluation of different fungicides were made in glass house maintained at 21± 2°C. Seeds of NP 830 susceptible to disease were sown in 25cm diameter earthern pots filled with sun-dried field soil. Fungicides were sprayed at the concentration of 0.25%. The first spraying was done when the seedlings were 30 days old, followed by two more applications at an interval of 15 days. After 24 hours of the final spraying operation of the fungicides were over, the treated plated plants were inoculated with the conidial suspension of *A. triticina*.

Control plants which received no fungicidal spray were also inoculated. Four replications were used in each treatment. The inoculated plants were kept in moist chamber (RH 80%) for 24 hours to facilitate infection after which they were allowed to grow under normal glass house condition. All the leaves from each replication were collected and infection values were determined according to the method described by Verma *et al* (1969).

Grade	*% of leaf area affected*	*Mean value*
Grade 0	0	0
Grade 1	1———10	5
Grade 2	11———30	20
Grade 3	31———50	40
Grade 4	51———70	60
Grade 5	71———90	80
Grade 6	91———100	95

Infection values (IV) were calculated by multiplying the number of leaves in grade 0, 1, 2, 3, 4, 5, and 6 by 0, 5, 20, 40, 40, 60, 80 and 95 respectively, summing all the grades. Thus formula used was as follows :

$$IV = \frac{\Sigma \text{(Number of diseased leaves in each grade X Mean value of each grade)}}{\text{Total number of leaves in all greades}}$$

RESULTS

Utilization of carbon sources : Eight different carbon compounds were tested. The equivalent amount of carbon present in 1% glucose was used as standard and added seperately to the basal medium (0.2% asparagine, 0.1% KH_2PO_4, 0.05% $MgSO_4$, $7H_2O$, 2 ppm Zn^{++}, Mn^{++} and Fe^{+++}). In the present study, cellobiose was found to be the best source of carbon representing maximum growth of mycelium. The growth of mycelium obtained with cellobiose was even better than that of other monosacharides used (Table 1).

Utilization of nitrogen sources : In this experiment the basal medium (1 % glucose, 0.1% KH_2PO_4, 0.05% $MgSO_4$, $7H_2O$, 2 ppm Zn^{++}, Mn^{++} and Fe^{+++}) was supplemented with a desired amount of nitrogenous compound. The amount of nitrogen present in 0.2 % asparagine was taken as a standard. Nitrogen was found to be effectively utilised by *A. triticina* when basal medium was supplemented with peptone and yeast extract indicating suitability of organic nitrogen sources in fungal growth (Table 2).

Free amino acid, sugar and organic acid composition of mycelium : Alcohol extract of the mycelium mat grown on Czapek-Dox medium (pH 6.0) at 30° for 15 days revealed presence of 13 amino acids, 4 sugars and 2 organic acids (Table 3).

Bio-assay of fungicides : Cent per cent inhibition could be obtained with vitavax (500 ppm) and dithane M-45 (2000 ppm). At 2000 ppm concentration 90 % and 86 % inhibitions were observed with bisdithane and blitox respectively indicating that vitavax is the most potent fungicide for controlling *A. triticina* (Table 4).

Disease control : Effective control of disease was achieved with vitavax and is followed by dithane M-45 and bisdithane (Table 5). The results presented in Tables 4 and 5 confirmed that vitavax is a potent fungicide for controlling leaf blight disease of wheat under both in vitro and in vivo condition.

Table 1, *Effect of different carbon sources on growth of* A. triticina

Carbon sources	Average dry mycelial weight (mg) *	
	5 days	10 days
D-Glucose	68.52	225.58
D-Fructose	60.63	206.05
D-Galactose	51.84	238.90
D-Mannose	64.12	286.18
D-Xylose	48.68	255.34
L-Arabinose	50.00	244.62
Sucrose	55.82	265.00
Cellobiose	69.35	306.58
Control (without carbon)	26.75	68.34

* Average of 4 replicates

Table 2. *Effect of different nitrogen sources on growth of* A. triticina

Nitrogen sources	Average dry mycelial wetght (mg) *	
	5 days	10 days
Inorganic sources		
Ammonium nitrate	45.58	160.00
Potassium nitrate	34.85	105.76
Calcium nitrate	41.64	155.37
Ammonium sulphate	36.36	70.68
Organic sources		
Yeast extract	38.35	238.75
Peptone	48.84	250.66
Urea	32.20	165.80
Control (without nitrogen)	12.64	34.58

* Average of 4 replicates

Tabie 3. *Free amino acid, sugar and organic acid composition in the mycelial mat of* A. triticina

Components	Ethanol extract
Amino acids	
Lysine	+
Histidine	+ +
Arginine	+
Aspartic acid	+ +
Serine	+
Glutamic acid	+
Glycine	+ +
Alanine	+
Valine	+
Leucine/Isoleucine	+
Tyrosine	+ +
Phenylalanine	+
Tryptophan	+
Sugars	
Glucose	+ +
Fructose	+
Mannose	+
Raffinose	+
Organic acids	
α-Keto gluteric acid	+
Succinic acid	+

Table 4, *Bio assay of different fungicides using growth inhibition study of* A. triticina *and LD50 values*

Fungicides	concentration in ppm					
	50	100	500	1000	2000	LD50 values
Captan	12.55 *	28.30	60.86	65.40	71.25	370.15
Blitox	8.00	35.66	78.40	84.58	86.20	231.80
Dithane M-45	26.82	64.52	82.35	89.67	100.00	78.56
Dithane Z-78	18.45	35.78	65.53	69.23	72.84	282.84
Vitavax	29.36	67.42	100.00	100.00	100.00	75.12
Bisdithane	21.58	62.41	80.65	86.42	90.16	86.43

Per cent inhibition in respect to control
Each data is an average of 4 replicates

Table **5.** *Infection value under different fungicidal treatment*

Fungicides (0.25% concentration)	Infection value
Captan	62.40
Blitox	51.33
Dithane M-45	30.47
Dithane Z-78	58.36
Vitavax	25.15
Bisdithane	32.40
Control (without fungicide)	74.55

DISCUSSION

Disaccharides like cellobiose and sucrose are best utilized even than that of their respective monosaccharide units. The disaccharides are directly utilized or their hydrolysed products are being utilised rapidly (Srivastava and Tandon, 1969; Kapoor and Tandon, 1971). Similar to *A. triticina*, slow utilization of sugars in the early stage which becomes more rapid with the bulk formation of mycelial mat has been reported in many other fungi (Masiza, 1968; Prasad and Choudhury, 1973).

Better growth by ammonium nitrate than other forms of nitrate is due to availability of nitrogen in the form of both (NH_4^+) and (No_3^-) ions as suggested by Malaca *et al* (1966). Similar to *A. triticina*, other fungi like *Colletotrichum coccoides* and *Colletotrichum gloeosporoides* also prefer nitrogen in the form of nitrate (Kurtz and Fergus, 1964). The priority of peptone over all other nitrogenous compounds could be attributed to their complex nature (Thind and Sandhu, 1956; Tandon and Chandra, 1962; Singh, 1966). Presence of large number of amino acids in the mycelial mat of *A. triticina* may be attributed due to its pathogenic nature.

The results of the trials of different fungicides under both in vitro and in vivo condition reveal that vitavax is the most effective fungicide and is followed by dithane M-45, bisdithane in controlling *Alternaria* leaf blight of wheat. In view of the efficiency of vitavax, it may be recommended for controlling leaf blight of wheat in areas where the disease is prevalent.

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