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PHYSIOLOGICAL STUDIES ON *COLLETOTRICHUM DEMATIUM* f. *TRUNCATA*, THE FUNGUS ANTHRACNOSE OF *PHASEOLUS MUNGO* LINN.

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Abstract

Conidia of *Colletotrichum dematium* f. *truncata*, the fungus isolated from diseased stems of *Phaseolus mungo* showed good germination in dextrose solution of different strengths as well as in distilled water, and 2% aquous solutions of sucrose and lactose. Its growth and sporulation was best after 15 days in basal agar medium containing sucrose and dextrose respectively. The minimum, optimum and maximum temperatures for its growth and sporulation were found to be about 20, 30 and 35 C. The nitrogen source best suited to this fungus was found to be ammonium nitrate. A pH of 4.8 was most suitable for the growth of the fungus. Its sporulation was best between a pH range of 6.1 and 7.8.

Introduction

Anthracnose diseases in leguminous crops are caused by species of *Colletotrichum* and related genera and often cause great deal of damage to the crop. The species of *Colletotrichum* found on legumes are *Colletotrichum lindemuthianum* (Sac. & Magn). Briosi & Cavara, *C. dematium* f. *truncata* (Schw.) Andrus & Moore, *C. villosum* Weimer, *C. graminicolum* (Cesati) G.W. Wilson. *C. trifolli* Bain & Essary and *C. destructivum* O'Gara (Tiffany and Gilman 1954).

Sattar and Hafiz (1952) reported an anthracnose disease of pulses ('mung' and 'mash') from West Pakistan which they considered to be cuased by *Gloeosporium phaseoli*. Ghafoor and Khan (1966 and 1967) reported *Colletotrichum dematium* f. *truncata* (which they called as *Colletotrichum truncatum*) on *Medicago sativum* from West Pakistan. A species of *Colletotrichum* was constantly found causing anthracnose of "mash" in the experimental area at West Pakistan Agricultural University, Lyallpur with the following characters.

Acervuli irregularly arranged, circular and oval, brownish black, 144-420 x 120-240 μ ; setae black, thick-walled, 2-3 septate, acute at the apex, 50-150 μ long and 3-4.3 μ wide at the base ; conidiophores straight, aseptate, hyaline ; conidia, hyaline, falcate with pointed ends, granulose, aseptate, 15.3-23.2 x 2.2-3.1/μ.

From a comparison of the morphology of this fungus with other species of *Collectotrichum* (Von Arx, 1957) it turned out to be *C. dematium* f. *truncata*.

Materials and Methods

The fungus was isolated from the diseased stems of *Phaseolus mungo* (mash) which were collected from the experimental farms of West Pakistan, Agricultural University, Lyallpur (LHL 1678). Stock cultures of the fungus were maintained on potato-dextrose agar (PDA) which were subcultured at regular intervals.

Composition of the media used in this study is given below :

Plain agar (PA) :	Agar, 20g ; distilled water to make one litre.
Potato dextrose agar : (PDA)	Potato starch, 20g ; dextrose, 20g ; agar, 20g ; distilled water to make one litre.
Malt extract agar : (MEA)	Pepton, 1g ; agar, 20g ; malt extract 20g ; dextrose, 20g ; distilled water to make one litre.
Corn meal agar : (CMA)	Corn meal, 30g ; agar, 20g ; distilled water to make one litre.
Basal medium : (BMA)	Dextrose, 20g ; potassium dihydrogen phosphate. 1.5g ; magnesium sulphate 0.5g ; potassium nitrate, 3.12g ; agar, 15g ; distilled water to make one litre.
Czepex agar : (CZA)	$NaNO_3$, 2.0g ; K_2 $HP0_4$ 1.0g ; KCl, 0.5g ; $MgS0_4$, $7H_20$, 0.5g; $FeSO_4$ 0.01g ; sucrose 30.0g ; agar 15.0g ; and water to make one litre.

Studies on the germination of fungus spores were carried out in "Van Tieghem cells".

All the cultural studies of the fungus were made in quadruplicates at controlled temperature of 30C unless otherwise mentioned.

Growth of the fungus in culture was measured in terms of colony diameter and the density of the growth was also taken into consideration in the interpretation of results. Two observations of colony dia were taken at right angle to each other for every Petri plate. Thus an average of eight readings was taken for the determination of colony diameter.

Dextrose in basal medium was replaced by sucrose, maltose, lactose and starch in order to study the effect of different carbon sources on the growth and sporulation of the fungus.

For studying the effect of different nitrogen sources on the growth and sporulation of this fungus, the amount of nitrogen was calculated in case of every nitrogen source to contain an amount of nitrogen equal to the amount present in 3.12 g of potassium nitrate.

Results and Discussion

Germination of conidia

The germination of conidia of *Colletotrichum dematium* f. *truncata* was studied in 0.0, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0% of sterilized aqueous solutions of dextrose, 2% sucrose and lactose solutions.

The percentage germination of conidia at 30 C after 24 hrs is given in Table 1.

TABLE 1 : *Effect of different concentrations of sugars on the germination of conidia of* Colletotrichum dematium *f.* truncata *at 30C after 24 hrs*

Solution in distilled water in per cent		Percentage germination of conidia
Dextrose	0.0	64.0
	0.5	65.0
	1.0	68.0
	2.0	85.0
	3.0	72.2
	4.0	69.4
	5.0	66.6
Sucrose	2.0	71.4
Lactose	2.0	71.4

In dextrose solution maximum germination of conidia took place in 2.0 per cent of the solution and the least in distilled water. There was a little difference in percentage of germination in distilled water and 5.0% dextrose solution. In another experiment the percentage of germination of conidia in 2.0 per cent dextrose solution was compared with the germination in sucrose and lactose solutions, however, 2.0 per cent dextrose solution showed an increase in percentage of germination of spores of about 14 per cent.

Growth and Sporulation

Effect of culture media

The colony diameter attained by the fungus on six culture media used indicated that the fungus grows well on all culture media. Maximum colony diameter was attained by the fungus on BMA, followed by PDA, CZA, MEA and CMA The growth of the fungus was least on PA. The growth of mycelium was dense on BMA, PDA, CZA and CMA. It was sparse on PA and MEA. Formation of acervuli was very high on PDA and CZA followed by MEA, BMA and CMA and low on PA (Table 2).

TABLE 2 : *Effect of different culture media on the colony dia of* Colletorichum dematium *f.* truncata *at 30C after 8 days*

Sr. no.	Culture media	Average colony dia (in mm)	Mycelial growth	Formation of acervuli	Formation of conidia
1.	Plain agar	38.5	Loose, white	Poor	Poor
2.	Malt extract	47.0	Loose, whitish gray	Good	Fair
3.	Basal medium	53.5	Dense, whitish gray	Good	Fair
4.	Potato dextrose agar	48.5	Dense, whitish pale and dark gray	Very good	Good
5.	Czepex agar	47.2	Dense, white gray	Very good	Good
6.	Corn meal agar	46.0	Dense, pallid mouse gray	Good	Fair

Effect of different sources of carbon

Twenty gms each of four different sources of carbon viz. sucrose, maltose, lactose and starch in place of dextrose were used separately in BMA in order to study their effect on the growth and sporulation of *Colletotrichum dematium* f. *truncata*. Observations taken 8 days after inoculations showed that the fungus attained maximum colony dia on maltose followed by sucrose and starch. The least growth of the fungus was on dextrose.

After 15 days of inoculation there was not much difference in the colony diameter of the fungus on four carbon sources, however, it attained its maxmium colony dia on BMA with sucrose as a source of carbon. This was closely followed by starch, dextrose and lactose. The least growth of the fungus was on maltose.

The growth of the fungus mycelium was dense and whitish on dextrose and it was sparse on other four sources of carbon.

The formation of acervuli was best on media containing dextrose and starch, less on maltose and least on sucrose and lactose. Formation of conidia was high on dextrose and starch and low on other three sources of carbon (Table 3).

TABLE 3 : *Effect of different sources of carbon on colony dia of the fungus* Colletotrichum dematium *f.* truncata *at 30C*

Sr. no.	Name of carbon source	Av. colony dia (in mm) after 8 days	Av. colony dia (in mm) after 15 days	Mycelial growth	Av. no. of acervuli per sq mm	Formation of conidia
1.	Dextrose	53.7	88.0	Dense, gray, mouse gray	1.86	Good
2.	Sucrose	62.0	92.0	Sparse white	0.75	Poor
3.	Maltose	64.0	82.0	Sparse, white	1.1	Poor
4.	Lactose	46.0	85.0	Sparse	0.5	Poor
5.	Starch	57.0	89.0	Sparse	1.2	Good

The results point out that the best source of carbon for growth is sucrose and for sporulation is dextrose in BMA medium. The growth was best on maltose till 8th day of the incubation, then it slowed down. After 8 days and till at the end of 15 days, there was least growth on maltose and best on sucrose. Mathur (1949) working with *Colletotrichum lindemuthianum* found that the fungus does not readily sporulate on common synthetic media, however, he found good sporulation on a special medium containing mineral salts, neopeptone, and glucose, sucrose, xylose or galactose as carbon sources. Our fungus sporulated readily on common culture media ; however, best sporulation was obtained on BMA medium containing dextrose.

Effect of different sources of nitrogen

The sources of nitrogen used in these studies were potassium nitrate, sodium nitrate, ammonium nitrate, ammonium sulphate and urea in BMA. Final observations on colony dia of the fungus grown on BMA with different sources of nitrogen were recorded after 8 and 15 days and are summarized in Table 4.

TABLE 4 : *Effect of different sources of nitrogen on colony dia of* Colletotrichum *f.* truncata *at 30C*

Sr. no.	Name of nitrogen source	Average colony dia (mm) after 8 days	Average colony dia (mm) after 15 days	Mycelial growth	Formation of acervuli	Formation of conidia
1.	Potassium nitrate	54.8	91.0	Medium	Very good	Good
2.	Sodium nitrate	58.3	86.3	Dense	Very good	Good
3	Ammonium nitrate	63.5	91.3	Dense	Very good	Good
4.	Ammonium sulphate	47.0	51.0	Sparse	Poor	Poor
5.	Urea	53.0	90.0	Sparse	Good	Fair

The growth of the fungus was maximum on BMA when ammonium nitrate was used as nitrogen source followed by sodium nitrate, potassium nitrate and urea after 8 days. The least growth of the fungus was attained when ammonium sulphate was used as a source of nitrogen in BMA.

The colony dia of the fungus was maximum after 15 days on BMA having ammonium nitrate followed by potassium nitrate, urea and sodium nitrate. The colony dia of the fungus was least on BMA having ammonium sulphate.

The growth of fungal mycelium was dense on BMA having sodium nitrate and ammonium nitrate followed by potassium nitrate. Mycelial growth was scanty on BMA having ammonium sulphate and urea.

The formation of acervuli was very good on BMA containing potassium nitrate, sodium nitrate, ammonium nitrate, good on urea and poor on ammonium sulphate. The formation of condia was high in all the cases of nitrogen sources except ammonium sulphate.

Mathur (1949) found best sporulation of *Colletotrichum lindemuthianum* when neopeptone was used as a nitrogen source. The other nitrogen sources in order of effectiveness were urea, glycine, arginine, asparagine and sodium nitrate.

He further found that his fungus was partially deficient for inositol and biotin. While, the fungus under the present studies did not show any deficiency for any nitrogen source, it grew and sporulated best in ammonium nitrate and the least in ammonium sulphate.

Effect of temperature

The fungus grew well over the temperature range of 25 to 30 C on basal agar medium and did not grow at all at 15 and 40 C. The maximum colony dia of the fungus was attained at 30 C. The least growth of the fungus at a higher temperature was at 35 C (Table 5).

TABLE 5 : *Effect of different temperatures on colony dia of* Colletotrichum dematium *f.* truncata *on basal medium*

Sr. no.	Tempera-ture in C	Av. colony dia (in mm) after 8 days	Av. colony dia (in mm) after 15 days	Mycelial growth after 15 days	Formation of acervuli	Formation of conidia
1	15	—	—	—	—	—
2	20	40.33	51.5	Medium	Poor	Poor
3	25	50.0	83.0	Good	Fair	Fair
4	30	53.2	91.0	Dense	Very good	Good
5	35	13.7	37.7	Sparse	Poor	Poor
6	40	—	—	—	—	—

Mycelial growth of the fungus was dense at 30 C followed by that at 25 C. The culture was fluffy and whitish. Formation of acervuli was high at 30C and to a lesser extent medium at 25 C. No acervulus was formed at 35 C. Cox (1949) found that the cardinal temperatures for *Colletotrichum truncatum* isolated from lima beans were in the range of 5, 27, and 35 C. Our results differ significantly only at minimum temperature, and somewhat at optimum temperature. This could be interpreted as an adaptation in the case of our strain to higher temperatures prevalent in our country.

Effect of pH

The growth of the fungus *Colletotrichum dematium* f. *truncata* was studied at 8 different levels of pH on BMA medium i.e. 3.6, 4.8, 6.1, 6.6, 7.2, 7.8, 8.4 and 9.0. Observations were made after 8 and 15 days of inoculation and are recorded in Table 6.

TABLE 6 : *Effect of different pH values on the growth of* Colletotrichum dematium *f.* truncata *at 30C*

Sr. no.	pH value	Av. colony dia (in mm) after 8 days	Av. colony dia (in mm) after 15 days	Mycelial growth after 15 days	Formation of acervuli after 15 days	Formation of conidia after 15 days
1	3.6	58.0	86.75	Sparse	Fair	Poor
2	4.8	69.0	88.75	Dense fluffy	Fair	Poor
3	6.1	65.0	84.5	Sparse	Very good	Good
4	6.6	63.0	82.5	Sparse	Very good	Good
5	7.2	62.0	82.0	Sparse	Very good	Good
6	7.8	61.0	82.5	Sparse	Very good	Good
7	8.4	61.5	80.5	Dense	Fair	Poor
8	9.0	58.0	78.0	Dense	Fair	Poor

The fungus grew well over the pH range of 3.6 to 9.0. Maximum growth of the fungus was attained at 4.8 after 8 and 15 days.

The minimum growth of the fungus was at pH 3.6 and 9.0 after 8 days and at 9.0 after 15 days.

Mycelial growth of the fungus was dense at pH 4.8, 8.4 and 9.0. Formation of acervuli was very high at pH 6.1, 7.2 and 7.8.

Mathur (1949) found that the best sporulation was between pH 5.2 and 6.5 in case of *Colletotrichum lindemuthianum*. Our fungus produced least number of acervuli and conidia below pH 6.1 and above pH 7.8. The best sporulation was obtained between pH range 6.1 to 7.8.

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