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Effect of Different Culture Media on Growth and Sporulation of *Alternaria brassicae* Incident of Alternaria blight of Mustard

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A B S T R A C T

Alternaria blight of mustard caused by *Alternaria brassicae* (Berk.) Sacc., is one of the major diseases of Indian mustard [*Brassica juncea* (L.) Czern & Coss.]. Among the eight tested media for growth of *A. brassicae*, Mustard leaf extract was recorded as most suitable for growth and sporulation in all the 20 isolates of *A. brassicae*. This was followed by Potato dextrose agar and Cabbage leaf extract medium. The maximum mycelial growth of 88.3 mm was recorded in Mustard leaf extract after 12 days of inoculation. The isolates were grouped into 4 categories based on growth and sporulation. In total 11 and 9 isolates were recorded as abundant growing on mustard leaf extract and potato dextrose agar medium respectively. However, all the 20 isolates were recorded as poor or slow growing on all the media except PDA and mustard leaf extract media. The minimum average mycelia growth of 32.28 mm was recorded in carrot agar medium after 12 days of inoculation which showed least supporting medium for growth of *A. brassicae* among the eight tested media.

Introduction

Rapeseed-mustard group is one of the important oilseed crops cultivated in India. India ranks first both in area and production of rapeseed and mustard in Asia (Anon, 2012). Mustard (*Brassica juncea* (L.) Czern & Coss.) is cultivated in an area of 6.70 million ha with a production of 7.96 mt and productivity of 1188 kg/ha. Rajasthan, Uttar Pradesh and Madhya Pradesh are the major rapeseed and mustard growing states in India. The production and productivity of rapeseed and mustard are hampered due to different biotic and abiotic challenges faced by the crop. Among various biotic factors, *Alternaria* blight caused by *A. brassicae* has been reported universally from all the continents of the world and thus omnipresent in nature.

Alternaria affects most cruciferous crops, including broccoli and cauliflower, field mustard and turnip, leaf or Chinese mustard, Chinese or celery cabbage, cabbage, rape, and radish. Hence, *A. brassicae* and *A. brassicicola* are cosmopolitan in their distribution. Symptoms of both these diseases on same leaves are quite common, while, combined infection of downy mildew and white rust on mustard have been observed

rarely. *Alternaria* blight causes up to 47 per cent yield loss in mustard (Meena *et al.,* 2010) with no proven source of resistance till date. *A. brassicae* usually sporulate poorly, and provide slow mycelial growth in common media particularly Potato Dextrose Agar (Meena *et al.,* 2012). *A. brassicae* is sensitive to nutritional and environmental factors and thus its growth and sporulation is influenced by composition of the nutrient media. Therefore, present study was carried out using a set of 20 different isolates of *Alternaria brassicae* from mustard for their preferential selectivity on eight different media to obtain better growth and sporulation.

Materials and Methods

Collection, isolation and purification of disease sample

During Dec.- Jan., of 2015-17 survey was conducted in mustard growing areas and markets of different divisions of Madhya Pradesh including Gwalior, Morena, Bhind, Datia, Sheopur and Shivpuri etc. and it was observed that there is severe infection of *Alternaria* blight in mustard. Special emphasis was given for occurrence and study of symptoms of the disease during different stages of plant growth at JNKVV, College of Agriculture, Jabalpur. *Alternaria brassicae* culture was isolated from diseased mustard plant showing concentric ring like symptoms on leaves from different areas. Isolates were identified microscopically by their characteristic shape of conidia. Isolates of the pathogen were maintained in culture tubes containing PDA medium and used as stock culture of the target organism throughout the study.

The leaves of affected mustard plants showing typical symptoms of *Alternaria* blight were cut (sterilized scalpel) and isolation were made for the presence or absence of the causal organism. These selected infected spots were washed 3-4 times in sterilized distilled water followed by surface sterilization by dipping in 4% NaOCl solution for 1 min, followed by washing with sterilized water 3-4 times. Surface sterilized pieces were then aseptically transferred into 9 cm Petri dishes containing Potato Dextrose Agar (PDA) and incubated at 25±2°C for seven days. Thereafter, growing mycelia from margin of apparently distinct colonies of the leaf spot pieces were aseptically transferred into another Petri plate containing PDA medium, where it was grown for 15 days at 23±2°C in the BOD incubator. On the basis of their conidiophore and conidial morphology as described by Simmons (2007), the pathogen was identified as *Alternaria brassicae* (Berk.) Sacc. and purified by single spore isolation method. The culture was preserved in the refrigerator (4°C) for further studies. The detailed location and coding of the isolates has been presented in table 1.

Results and Discussion

All the eight tested media significantly supported the growth of *A. brassicae*. However, isolates showed selectivity for its growth on different tested media. The maximum mycelia growth of 88.33 mm was attained on mustard leaf extract. Average mycelia growth of 20 isolates ranged from 32.28 mm (Carrot Agar medium) to 76.10 mm on mustard leaf extract medium. Among the 20 different isolates, one isolate named I13 showed maximum mycelia growth of 87.0 mm and 88. 3 mm on PDA and mustard leaf extract medium respectively. However, on other media different isolates showed different preferential reactions for its mycelia growth. One isolate namely I12 was recorded as slowest in its mycelia growth on four different media namely PDA (50.0 mm), Mustard leaf extract (55.3 mm), Cabbage leaf extract (38.6 mm) and cauliflower leaf extract media 931.3

mm). However isolate I18 showed least mycelia growth on Corn meal Agar (36.3 mm), Czepexdox Agar (45.3 mm) and Rose Bengal Agar medium (38.0 mm). This showed significant variability among the 20 tested isolates of *A. brassicae* and their preferential selectivity in utilizing the media for their growth. The detailed data of 20 isolates of *A. brassicae* on eight different media have been presented in table 2.

Based on their growth on different media, the isolates were categorized into four classes including poor, slow, good and abundant growth type. It was observed that maximum number of 11 isolates could be categorized under abundant growth type class on Mustard leaf extract media. This was followed by 8 isolates on PDA showing abundant growth. In total, 9 and 7 isolates were grouped under good growth type class on PDA and mustard leaf extract medium. Further, all the 20 isolates showed poor type growth on Carrot agar medium. This was followed by 19 isolates of poor growth type on Capexdox agar medium. It was observed that 100% isolates fell into either poor or moderate mycelia growth type class on all the media except PDA and mustard leaf extract medium. Among the 20 different isolate one isolate showed poor and moderate growth on PDA. However, on mustard leaf extract, only two isolates showed moderate type mycelia growth. This indicated, the PDA and mustard leaf extract medium to be better growth supporting media for *A. brassicae*. The grouping of all the 20 isolates of *A. brassicae* falling in different classes of mycelia growth has been presented in table 3.

Table.1 List of *Alternaria brassicae* isolates collected from different locations

S. No.	District	Location	Previous crop	Variety	Isolates code
1.	Gwalior	Gwalior	Bajra	Varuna	I_1
		Bhitarwar	Jowar	Rohini	I_2
2.	Morena	Morena	Bajra	Varuna	I_3
		Abhah	Bajra	NRC-2	I_4
		Porsa	Jawar	Kranti	I_5
		Joura	Bajra	Kranti	I_6
3.	Bhind	Bhind	Jawar	JM -3	I_7
		Lahar	Urd	JM -3	I_8
		Gohad	-	JM-3	I_9
		Atair	sesame	Urvasi	I_{10}
		Mehgoan	Bajra	Arpan	I_{11}
4.	Datia	Datia	Sesame	Varuna	I_{12}
		Seondha	-	Varuna	I_{13}
		Bhander	Sesame	Varuna	I_{14}
5.	Sheopur	Sheopur	Urd	NRC-2	I_{15}
		Karahal	Soybean	JM-3	I_{16}
		Vijaypur	-	Varuna	I_{17}
6.	Shivpuri	Pohri	-	Kranti	I_{18}
		Karera	Urd	Varuna	I_{19}
7.	Jabalpur	Jabalpur	Soybean	Pusa bold	I_{20}

Table.2 Effect of different media on mycelial growth of 20 isolates 0f *Alternaria brassicae*

Isolate	Mycelial growth (mm) 12 days after inoculation							
	Potato dextrose agar	Mustard leaf extract	Cabbage extract	Caulifl ower extract	Carrot agar	Corn meal agar	Czapex dextrose	Rose Bengal agar
I_1	81.3	81.6	45.3	42.0	39.3	40.6	25.3	35.0
I_2	85.0	86.3	51.3	35.6	30.6	39.6	31.6	43.6
I_3	80.0	80.6	38.6	41.3	34.3	43.6	36.0	35.3
I_4	75.0	77.0	52.3	35.3	38.6	48.6	40.6	41.3
I_5	74.0	75.3	58.6	51.3	25.6	54.6	37.6	45.3
I_6	76.0	78.0	55.3	41.6	33.3	44.6	51.6	35.6
I_7	72.0	74.3	53.6	38.6	43.0	37.3	35.0	45.6
I_8	69.6	71.0	48.6	40.6	40.6	39.3	31.3	51.6
I_9	55.3	60.6	54.0	44.3	35.6	52.3	41.3	52.3
I_{10}	70.0	72.3	50.3	52.0	31.3	47.6	49.0	53.6
I_{11}	71.0	72.0	45.3	41.3	26.3	47.0	26.0	35.3
I_{12}	50.0	55.3	38.6	31.3	32.0	45.0	22.6	38.0
I_{13}	87.0	88.3	52.0	48.6	27.6	41.0	31.6	35.3
I_{14}	80.0	83.6	55.6	48.0	36.6	43.6	43.0	38.3
I_{15}	78.0	78.3	45.6	41.3	38.0	49.6	30.0	34.0
I_{16}	85.0	86.3	50.0	52.0	34.3	50.6	29.0	40.6
I_{17}	83.0	84.3	55.6	56.3	25.0	50.0	38.3	45.0
I_{18}	73.0	75.3	59.3	52.0	26.6	36.3	45.3	38.0
I_{19}	75.0	76.3	58.3	52.6	23.6	39.0	22.3	31.3
I_{20}	62.0	65.3	51.3	46.3	23.3	42.0	22.6	37.3
Avg..	74.11	76.10	50.98	44.62	32.28	44.62	34.50	40.63
Sem±	**0.2**	**0.3**	**0.4**	**0.5**	**0.5**	**0.5**	**0.6**	**0.6**
CD at 5%	**0.5**	**1.0**	**1.4**	**1.6**	**1.5**	**1.5**	**1.9**	**1.7**

Table.3 Categorization based on rate of mycelial growth

Category	PDA	MLE	CBLE	CLE	CA	CMA	CzDA	RBA
0	1	00	8	14	20	18	19	17
I	1	2	12	06	00	02	01	03
II	9	7	00	00	00	00	00	00
III	9	11	00	00	00	00	00	00

Category – 0 (Poor), Category-I (Moderate), Category- II (Good), Category- III (Abundant)

Table.4 Effect of different media on sporulation of 20 isolates of *Alternaria brassicae*

Isolate	PDA	MLE	CBLE	CLE	CA	CMA	CzDA	RBA
I_1	++	++++	+++	++	++	+	++	++
I_2	++	+++	++	++	++	+	+	+
I_3	++	+++	++	++	++	+	+	+
I_4	++	++++	++	+++	++	-	+	++
I_5	++	++	++	++	-	+	+	++
I_6	++	+++	+++	++	++	++	+	+
I_7	++	++	+++	++	+	+	++	+
I_8	++	+++	++	++	+	-	+	++
I_9	++	+++	++	++	-	+	+	+
I_{10}	+++	++++	++	++	+	+	+	+
I_{11}	+	+++	++	++	+	+	+	+
I_{12}	++++	+++	++	++	+	-	+	+
I_{13}	+++	+++	++	+++	+	+	+	++
I_{14}	++	+++	++	++	+	+	++	+
I_{15}	+++	+++	++	++	-	++	+	+
I_{16}	++	+++	++	+++	+	+	+	++
I_{17}	++	+++	++	++	+	-	+	+
I_{18}	++	+++	++	++	+	+	+	+
I_{19}	++	++	++	++	-	+	+	+
I_{20}	++	++	++	++	+	-	+	++

++++ = >30 conidia per microscopic field +++ =20-30 conidia pr microscopic field
++ = 10-20 conidia per microscopic field + 0-10 conidia per microscopic field
- = no sporulation

All the 20 isolates were sporulating on all the tested media except few isolates which were recorded as non-sporulating on carrot agar and Corn meal Agar medium. The degree of sporulation varied from isolate to isolate and media to media. However, most of the isolates produced abundant conidia on potato dextrose agar and mustard leaf extract medium. The conidial count for different isolates per microscopic field on different media has been presented in table 4.

Kumar and Singh (2003) and Singh *et al.,* (2015) conducted their studies on *A. brassicae* and they observed that there was presence of profuse variability among the isolates of *A. brassicae* on different media with respect to cultural and morphological characterization. They recorded that mycelia growth of *A. brassicae* was best supported by PDA followed by Radish dextrose agar and *Brassica* leaf extract agar media. The results obtained in present findings are in same fashion. However, Selvamani *et al.,* (2013) observed that Cauliflower Leaf Extract Agar was the best medium followed by Potato Dextrose Agar for mycelia growth. Mehra *et al.,* (2017) tested eight different nutrient media which also evidenced for variation in the radial growth of twenty different *A. brassicae* isolates as in the present finding.

The results of present findings are in confirmation with Shakya, 2012 who reported that mustard Leaf Agar medium was more appropriate for the culture of Alternaria blight pathogen over three tested media. Ansari *et al.,* (1988) reported that *Alternaria brassicae* sporulates well on range of media but maximum growth was recorded in PDA.

Mehta and Sangwan (2003) reported that Mustard Leaf Extract media was most favoring media for growth and sporulation of *A. brassicae* which are in same line as per results of present findings.

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