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Research Article

VARIABILITY OF CULTURAL MEDIA AND PH OF *ALTERNARIA* SPP PATHOGENIC TO DIFFERENT CROPS

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Abstract- *Alternaria* belonging to ascomycota subdivision of fungi causes most common and devastating disease in different crops. The maximum growth was observed potato dextrose agar as well as Oat meal agar media *in-vitro*. The maximum growth and sporulation of *Alternaria* spp., was obtained on potato dextrose agar media followed by Oat meal agar media *in vitro*. Seven media were used for testing the fungus growth and sporulation and compared. The growth characteristics such as colony color and substrate with the sporulation of the test fungus were observed. Maximum growth of mycelium was observed on PDA media at pH 6.0 and followed by 7.0 while maximum sporulation was observed at 7.0 followed by 6.0. Maximum growth of the fungus was observed at 8 days after inoculation with continuous increasing growth in the potato dextrose Agar medium, although the growth rate was decreased after the 2 days of inoculation. This study will be helpful for further investigations on the physiology of the fungus and management of the disease. This investigation may be useful for taxonomic study of the fungus.

Keywords- *Alternaria* spp., Media, pH and fungal sporulation

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Introduction

Alternaria species are known as major plant pathogens having 299 species in the genus. Preliminary reports on variability in *Alternaria* species were made from Holland [1] and UK [2]. *Alternaria* is ubiquitous in the environment and is a part of fungal flora almost present in nature. Which in case is normal agents of decay and decomposition. Many species are saprophytes commonly found in soil and Play a key role in decaying plant tissue, wood tissue, wood, wood pulp compost and sewage of jet fuel. Some species cause a wide range of disease with crops losses of cereal, vegetable, fruit, oil yielding and ornamental crops and also cause extensive spoilage of agricultural output as post-harvest pathogen [3]. The conidial spores are airborne and found in the soil and water, as well as in surrounding. The club-shaped spore are single form long chains type structure. They are able to grow on thick colonies, which are usually green, black or gray in colour. At least 20% of agricultural commodities spoilage is caused by *Alternaria* species. Most severe losses may reach up to 80% of yield. However species of the fungal genus are often prolific producers of a variety of toxic compounds. They effect most of these compounds have on plant health are not well explained in nature. Colonies are fast growing, black to olivaceous- black or grayish, and are seudo-like to floccose. Microscopically, branched acropital chains of multi-cellular conidia are produced sympodially from simple, sometimes branched, short or elongate conidiophore. Conidia are obclavate, obpyriform, sometimes ovoid or ellipsoidal, often with a short cylindrical beak, pale brown.

Materials and Methods

Cultural characters

For cultural characters the pathogen was studied on PDA medium. The Five mm disc of pure culture isolate was inoculated at the center of the poured petri plates from ten days old actively growing culture. All inoculated plates were incubated at 28±2°C in BOD incubator. The growth rate, colony characters and growth habit were visually observed after 48 hours of incubation till the complete growth of the pathogen in petri plates.

Effect of media on mycelial growth and sporulation of *Alternaria* spp.

The efficacy of various synthetic and semi-synthetic media on growth of *Alternaria* spp. was studied. Seven media *viz.* Oat meal agar, Richard's agar, Potato dextrose agar, Carrot agar, Rose bengal agar, Asthana and Hawker´s and Czapek-Dox agar were used [4]. The different types of media were prepared as per the composition and placed in 250 ml conical flasks. The different types of media were then poured in the sterilized plates @ 20 ml per petri plate uniformly and allowed to solidify. Mycelial disc of five mm diameter was cut from 7 days old culture of pathogen and was inoculated in the centre of each plate. The inoculated plates were incubated at 28±2°C till the pathogen completely occupied the plate. Three replications were maintained for each media. An observation on radial growth was recorded in cross way at 48 hours intervals till the completion of growth of pathogen (90 mm) in any plate. Colony characters and sporulation were also recorded at the end.

Effect pH on mycelial growth, sporulation and spore germination of *Alternaria* spp.

Potato Dextrose Agar media was used to study the effect of pH on the growth of *Alternaria.* Spp at different pH levels from 6.0 to 8.0 with the difference of 0.5 were adjusted with Citrate phosphate buffer and N/10 HCL or N/10 NaOH. Three replications were maintained, the inoculated plates were kept at 28±2°C for 8 days and observations on colony diameter were recorded at 48 hours interval and sporulation was recorded in the end of the experiment. In this way pH for good growth and sporulation of the fungus was determined.

Result and Discussion

Alternaria spp. showed variability in colony growth of the fungus on seven different media *viz.,* Potato Dextrose Agar, Czapeks dox, Carrot Agar, Oatmeal Agar Rose Bengal agar, Richard's agar, Asthana & Hawkers media and Carrot Agar media. The variability in growth was studied on different days after inoculation up to 10 days. Isolates showed significant difference recorded i.e. colour, colony diameter and days required for sporulation.

The effect of different media on mycelial growth of *Alternaria* spp. revealed that *Alternaria tenussima* (potato) exhibited a maximum mycelial growth on oat meal agar media while, minimum on Asthana & Hawker media, for tomato and cumin maximum mycelial growth was seen on PDA media and minimum on Asthana & Hawker media, for chilli, brinjal and dhatura maximum mycelial growth occurred on Oat Meal Agar Media while, minimum on Asthana & Hawker media, for onion and cauliflower maximum mycelial growth occurred on PDA media while, minimum on Rose Bengal Media and for sesame maximum mycelial growth occurred on PDA media while, minimum on Asthana & Hawker media, respectively.

Table-1 *Effect of different media on mycelial growth of Alternaria spp. isolate(mm)*

Isolate	Crops	Oat meal agar	Rechards Agar	Potato dextrose agar	Carrot Agar	Rose bengal	Asthana & Hawker	Czapek dox
Alternariatenussima	Potato	36.655.	31.70	33.20	31.40	29.28	18.85	30.43
Alternariasolani	Tomato	51.01	35.04	56.23	49.89	35.90	32.48	49
Alternaria alternate	Chilli	42.426.	28.87	39.15	37.56	37.56	23.32	30.68
Alternariaporri	Onion	48.60	40.39	50.82	46.14	30.25	38.34	42.70
Alternariabrassicae	Cauliflower	47.34	45.82	49.32	32.23	26.15	41.82	41.84
Alternaria sesame	Sesame	51.427.	42.76	54.04	39.40	32.76	29.57	37.76
Alternariamelongenaei	Brinjal	53.357.	40.81	50.39	48.37	31.78	19.56	46.65
Alternariatenussima	Dhatura	60.10	31.18	57.65	53.39	32.45	13.96	54.35
Alternariaburnsii	Cumin	55.427.	28.39	58.54	49.18	31.53	26.14	33.76

Table-2 *Table of CD, SE(d) and SE(m)*

Factors	C.D	SE(d)	SE(m)
Factor(A)	0.613	0.312	0.221
Factor(B)	0.541	0.275	0.195
Intraction A x B	1.622	0.826	0.584
Factor(C)	0.409	0.208	0.147
Intraction A x C	1.226	0.624	0.441
Intraction B x C	1.082	0.550	0.389
Intraction A x B xC	3.245	1.651	1.168

Table-3 *Effect of different media on sporulation of Alternaria spp (mm)*

Isolate	Crops	Oat meal agar	Rechards Agar	Potato dextrose agar	Carrot agar	Rose bengal	Asthana & Hawker	Czapek Dox
Alternariatenussima	Potato	++++	+	++	+	+	+	++
Alternariasolani	Tomato	++++	+	++	+	+	++	++
AlternariaAlternariaalternata	Chilli	++	+	++	+	+	+	++
Alternariaporri	Onion	++	+	++	-	+	-	++
Alternariabrassicae	Cauliflower	++	++	++	+	+	+	+
Alternariasesame	Sesame	++++	+++	++++	++	++	++	+++
Alternariamelongenae	Brinjal	++	++	++	+	+	+	+
Alternariatenussima	Dhatura	+	+	+	+	+	+	+
Alternariaburnsii	Cumin	++	++	++	+	+	+	+

- = No sporulation (0 spore/ microscopic field) ,
+ = Poor (0-10 spores/ microscopic field)
++ = Moderate (10-50 spores/microscopic field)
+++ = Good (50-100 spores/ microscopic field)
++++ = Abundant (more than 100 spores/microscopicfield)

Table-4 *Cultural characteristics of Alternaria spp on different media*

Isolate	Crop	Different Media						
		Oat meal agar	Rechards Agar	Potato dextrose agar	Carrot agar	Rose bengal	Asthana & Hawker	Czapek Dox agar
Alternariatenussima	Potato	Whitishto black green	Whitish black	Whitish black	Whitish black	Whitish black	Whitish –whitish black	Whitish-whitish black
Alternariasolani	Tomato	Dark olivacus gray	Dark olivacious	Black smoky	Black smoky	Black green	White gray	White gray
Alternariaalternata	Chilli	Gray brown	Gray brown	Gray brown	White brown	Whitish brown	Whitish brown	Whitish Brown
Alternariaporri	Onion	Greenish gray to black	Whitish black	Whitish to green gray	Blackish	Blackish white	Whitish gray colour	Greenish white cottony
Alternariabrassicae	Cauluiflower	Blackish	Blackish white	Blackish white cottony	Blackish white cottony	White black	White black	White black
Alternariasesami	Sesami	Darty white	Whitish growth	Whitish growth	Whitish growth	Whitish growth	Whitish growth	Dirty white
Alternariamelongenae	Brinjal	Whitish greenish with white	White brownish	Cotony growth	Whitish green	Whitish brown	Whitish brown	Whitish brownish with gray
Alternariatenussima	Dhatura	Cottony or valvaty	Pale gray	Cottony or valvaty	White black	White black	White black	White black
Alternariaburnsii	Cumin	Dirty green white	Greenish white	Greenish white	Dirty Greenish white	Greenish white	Greenish white	Greenish white

The effect of *Alternaria* sp. isolates obtained from different crops exhibited a wide range of sporulation pattern that is for potato abundant growth occurred on oat meal agar media, moderate on PDA and czapak dox media while, poor on rest other media. For tomato good growth was seen on oat meal agar media, moderate growth on PDA, Asthana and Haekers and czapak dox agar media while it showed poor growth on other media, for chilli moderate growth was seen on oat meal agar media, for onion maximum sporulation occurred on oat meal agar media, PDA and Czapak dox media. While poor and no sporulation was seen on Rechard agar rose, Bengal agar and carrot agar, Asthana and Hawkers media, respectively. Moderate sporulation of cauliflower brinjal and cumin was seen on oat meal agar, Rechards agar and PDA while, they recorded poor estimate of sporulation on rest other media. Dhatura was only the crop whose isolates exhibited poor sporulation on every media. Similar finding has been reported by [5, 6] for brinjal,chilli and potato isolate of *Alternaria* spp.

The effect of different PH on mycelial growth of *Alternaria* spp. isolates on different crops reveled that maximum and minimum growth for potato occurred at pH (6.0 and 8.0), for tomato (6.0 and 8.0), for chilli (7.5 and 8.0), for onion (6.5 and 8.0), for cauliflower, seasmi, Brinjal, Dhatura and cumin (6.0 and 8.0), respectively. It was seen that isolates of *Alternaria* spp. obtained from most of the crops exhibited maximum and minimum mycelial growth at pH 6.0 and 8.0 respectively.

Similar result has been obtained [7-9] for pH ranging between 5.5 to 7.2. The data on sporulation also suggested that maximum sporulation occurred at pH 7.0 which has also been reported by [9].

In general, the overall effect of isolate of *Alternaria* spp. for sporulation and mycelial growth was seen at pH 7.0 and 7.5.

Table-5 *Effect of different pH on mycelial growth of Alternariaspp isolate*

Isolate	Crops	pH				
		6.0	6.5	7.0	7.5	8.0
Alternariatenussima	Potato	48.04	34.5	35.12	38.79	16.04
Alternariasolani	Tomato	56.20	43.79	54.54	40.20	23.41
Alternariaalternata	Chilli	42.37	37.41	39.62	49.33	33.29
Alternariaporri	Onion	43.37	46.04	39.91	21.33	15.5
Alternariabrassicae	Cauliflower	62	47.70	52.54	43.54	32.45
Alternaria sesame	Sesame	58.16	46.45	55.75	46.95	39.83
Alternariamelongenae	Brinjal	54.75	51.45	48.37	48.91	34.04
Alternariatenussima	Dhatura	61.16	57.75	49.33	52.95	49.04
Alternariaburnsii	Cumin	60.70	58.5	46.91	38.41	28.20

Table -6 *Table of CD, SE(d) and SE(m)*

Factors	C.D	SE(d)	SE(m)
Factor A	8.693	4.42	3.125
Factor B	N/A	3.294	2.33
Intraction A x B	N/A	9.883	6.989
Factor C	5.795	2.947	2.084
Intraction A x C	N/A	8.84	6.251
Intraction B x C	N/A	6.589	4.659
IntractionAxBxC	N/A	19.76	13.977

Conclusion

In present study *Alternaria* species is isolated from the different hosts *viz.* Potato, Tomato, Chilli, Brinjal, Cauliflower, Sesame, Cumin, Onion and Dhatura. Seven media used to observe the growth and sporulation. Growth and sporulation also test at different pH. Potato dextrose media was found best for growth and sporulation of different *Alternaria* spp. Maximum growth was found at 6.0 pH of media followed by 7.0, while sporulation was found at 7.0 pH followed by 6.0.

Conflict of Interest: None declared

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