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Studies on *Alternaria porri* (Ellis) Ciferri pathogenic to Onion (*Allium cepa* L.)

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ABSTRACT

An attempt has been made to identify and study the growth pattern of Alternaria porri that causes *purple blotch of onions. Czapek-Dox medium amended with nutrients like lactose, urea, diammonium hydrogen orthophosphate, ammonium sulfate and pH 5.0 supported good growth of the pathogen. Toxic potential of the culture filtrates of the strain was assessed in terms of phytotoxicity against seed germination as well as seedling growth of onions and antibacterial activity against selected bacteria. Mancozeb and extracts from the plant species, Mentha arvensis were effective in controlling the growth of A. porri.*

Key words: *Alternaria porri*, onion, toxicity, fungicides, plant extracts.

INTRODUCTION

Vegetables are important as essential building blocks of any diet. Not only, they are loaded with vitamins and minerals which are essential for healthy living, but also constitute a part of balanced diet. Among the vegetables, onion (*Allium cepa L.)* often called as “queen of kitchen” is one of the oldest known and an important vegetable crop grown in India. It is commonly used for cooking purposes by almost all the people. It ensures excellent taste to dishes and also exhibits a number of therapeutic properties such as antibacterial, antifungal, antihelmintic, anti-inflammatory, antiseptic, antispasmodic, etc. [1].

Due to their enormous commercial and medicinal value, onions are cultivated in almost all countries of the world and consumed across the globe. Although, India is the largest producer of vegetables in the world, the productivity is very low at12.5 tonnes/ha as against to 15.8 tonnes/ha in China and 44.21 tonnes/ha in Japan [2]. Though onion is considered as an important fresh market vegetable and medicinal crop, it shows susceptiblibilty to numerous foliar, bulb and root pathogens that ultimately reduce yield and quality. Purple blotch of onion

caused by *Alternaria porri* is an important destructive disease of onion worldwide. Therefore, an attempt has been undertaken to isolate, identify and to study the growth pattern of the pathogen causing purple blotch in the onion fields. The effect of toxic metabolites produced by the fungus on germination and growth of onion was studied. In addition, control of the pathogen with fungicides and plant extracts was also evaluated.

MATERIALS AND METHODS

The infected leaves collected from onion fields of Kaza, located near Acharya Nagarjuna University Campus, Guntur were surface-sterilized with 0.1% mercuric chloride for one minute, rinsed thoroughly with sterilized distilled water, blotted to dry and then placed on Czapek-Dox (CD) agar medium. Cultural characteristics of the fungus isolated from the infected leaves were studied and tentatively identified it as *Alternaria porri* [3] according to standard protocols. Pathogencity of the fungus was proved by employing Koch's postulates and pure culture was maintained on CD agar medium.

Growth pattern of *Alternaria porri* in Czapek-Dox broth

Growth pattern of *A. porri* was determined by culturing the fungus in CD broth and the dry weight of the mycelium was recorded [4]. Autoclaved medium was inoculated with the mycelial disc of 5 mm diameter, taken from the periphery of actively growing culture using sterilized cork borer. The flasks were incubated at room temperature (28±2 ^{o}C). At three-day intervals starting from the 3rd day after inoculation, the mycelial mats were collected by filtration through Whatman No.1 papers, dried at 60 ^{o}C for about 14 h and then weighed in three replicates.

Growth of *A. porri* at different levels of pH

To test the influence of pH on fungal growth, *A. porri* was cultured in CD broth initially adjusted to different levels of pH ranging from 2.0 to 9.0 using dilute acid or alkali [5]. Dry weight of the mycelium was recorded after 20 days of incubation.

Effect of nutrient sources on growth of *A. porri*

CD broth was used as a basal medium to study the effect of various nutrients such as carbon, nitrogen, phosphorus and sulfur compounds on growth of *A. porri* [4]. The seeded flasks were incubated for 20 days at room temperature as stationary cultures. Dry weight of the mycelial mats was recorded in terms of mg/100 ml.

Screening of *A. porri* for toxin production: *A. porri* isolated from the infected leaves of onion was screened for its toxigenic potential, taking phytotoxicity and antibacterial activity as the test criteria.

Phytotoxicity of culture filtrates of *A. porri*

To test the phytotoxic activity, the fungus was cultured on CD broth for 30 days. At the end of incubation, the culture filtrates were collected under aseptic conditions and tested for phytotoxicity against seed germination and root as well as shoot elongation of onions [6].

Screening of *A. porri* for the production of antibacterial compounds

The fungal isolate was cultured on yeast extract sucrose medium (YES) for 30 days at room temperature as stationary culture. At the end of incubation, the fungal mat was separated by

filtration through Whatman No.1 filter paper and the filtrates from all the three replicates were pooled. The culture filtrate was extracted with diethyl ether. The extract was evaporated to dryness on a water bath avoiding excess heat. The residues were dissolved in 1.0 ml of diethyl ether, transferred to a glass vial and preserved for testing antibacterial activity against selected bacteria such as *Bacillus megaterium*, *B. subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *P. fluorescens*, *Serratia marcescens* and *Xanthomonas citri* by using seeded plate technique [7]. The area of the inhibition zone was recorded after 24 h of incubation at room temperature.

Effect of fungicides against *A. porri in vitro*

The effect of five fungicides on the growth of *A. porri* was studied with a view to select an effective fungicide. Poison food technique as described by Grover and Bansal [8] was employed for evaluating the effect of fungicides like mancozeb, carbendazim, blitox, captafol and benlate under *in vitro* conditions. CD broths were prepared with different concentrations of selected fungicides varying from 10 to 500 ppm followed by inoculation with actively growing culture of *A. porri*. Medium without a fungicide served as control. Dry weight of the mycelial mats was recorded in three replicates at the end of 20 days incubation.

Efficacy of plant extracts against *A. porri*

To test the efficacy of different plant extracts on the growth of *A. porri*, the fungus was cultured on CD broth supplemented with different plant extracts in different concentrations [9]. Leaves from different plants were collected, washed thoroughly with sterilized distilled water, blotted and 100 g of leaves were ground with 100 ml of water. Leaf extract was filtered through muslin cloth. The filtrate was made up to 100 ml by with sterilized distilled water.

CD broth was prepared, sterilized and the plant extracts were added to basal medium aseptically, so as to get the required concentration of 10%, 25% and 50% for each plant extract. Flasks were inoculated with 5 mm mycelial discs of actively growing culture of *A. porri*. After 20 days of incubation, dry weight of the mycelial mats was recorded by conducting the experiment in three replicates.

RESULTS AND DISCUSSION

The infected leaves were observed for purple blotch symptoms. On infected leaves, small, sunken, oval to foot-ball shaped lesions were found. The lesions are brown to purple at the centre surrounded by a light brown area. Concentric light and dark zones are also observed on the infected leaves. In severe cases, blotches are enlarged up to 4 inches long and are covered with conidia. Brown lesions with reddish-purple margins resembling bull's-eye were also noticed. The symptoms were characteristic to that of purple blotch disease caused by *Alternaria porri* [10-11].

The pathogen was isolated from the infected leaves of onion and cultured on CD agar medium. The young hyphae were hyaline, slender, radiating and septate. The white colonies turned purple color with advancing age of culture. The conidiophores arose singly or in groups and were pale brown, erect, simple, cylindrical, septate. Conidia were 100-300 µm long, 15 to 20 µm thick, solitary, straight or curved with the body of conidium ellipsoidal tapering to the beak and having 7 to 9 transverse septa and 1 to 3 longitudinal septa. With the above characteristics, the pathogen

was identified as *Alternaria porri* in accordance to the report of Ellis [3]. The pathogenecity of the fungus was established by following Koch's postulates.

Growth pattern of *A. porri* in Czapek-Dox broth

Data on the growth pattern of *A. porri* cultured in CD broth for 27 days are recorded (Fig 1). Growth of *A. porri* was initially slow and the mold exhibited exponential growth between 6 and 20 days after inoculation. Thereafter, stationary phase extended from 21 to 27 days of incubation.

Influence of pH on the growth of *A. porri*

Influence of pH on the fungal growth was studied by culturing the strain in CD broths initially adjusted to different pH levels ranging from 2.0 to 9.0. Maximum fungal growth was observed in broth adjusted to pH 5.0 followed by the medium with a pH of 4.0 and 6.0. The growth was poor at pH 9.0. The fungus did not grow at highly acidic levels of pH 2.0 and 3.0 (Fig 2). Growth of *A. porri* was found maximum at pH 5.0 which is in conformity with the report of Ramamohana Rao and Vijayalakshmi [4] who observed good growth of *Alternaria* species between pH 4.0 and 6.0.

Effect of nutrient sources on growth of *A. porri*

A critical and comprehensive knowledge of nutritional patterns and factors influencing the growth of fungi is a prerequisite for any study leading to the understanding of host-pathogen relationship. Very little information is available on the cultural and nutritional parameters of *A. porri*. Hence, the influence of cultural and nutritional sources such as carbon, nitrogen, phosphorus and sulfur on growth of *A. porri* isolated from onion leaves was determined.

In plants, carbohydrates are available in simple as well as in complex form and fungi convert the complex forms into simple water soluble sugars of low molecular weight before utilization. It has been shown that different fungi respond differently with a particular compound and the fungus exhibited marked variation in the utilization of different carbon sources. Among the carbon sources tested lactose supported the best growth of *A. porri* followed by galactose and dextrose (Table 1). Fungal growth was poor when xylulose and maltose were used as carbon sources. . In contrast, Singh [12] reported sucrose as the best carbon source for the growth of *A. porri*. Therefore, the utilization of carbon sources by the fungi was found to be strain specific.
Nitrogen plays an important role in the nutrition of fungi. Hence, the growth of *A. porri* was tested with different nitrogen sources. The fungus exhibited good growth when cultured in CD broth with urea as nitrogen source followed by magnesium nitrate. Potassium nitrate supported moderate growth. Growth was very poor when ammonium nitrate or barium nitrate was used as nitrogen sources. Urea was found as the best nitrogen source for the growth of *A. porri* which was also proved by Hossain *et al* [13] from their findings.

Study of the effect of different phosphorus sources on growth of *A. porri* indicated diammonium hydrogen ortho phosphate as suitable ones followed by ammonium dihydrogen orthophosphate and disodium hydrogen orthophosphate. Moderate growth was recorded with zinc phosphate and sodium dihydrogen orthophosphate. Among five sulfur sources tested, ammonium sulfate and manganous sulfate supported good growth of the fungus followed by sodium sulfate. Growth was least with aluminium sulfate and potassium sulfate as sulfur sources.

Utilization of phosphorous and sulphur sources by *Alternaria* species was found to vary. Good growth of *A. sesami* was observed with disodium hydrogen phosphate followed by sodium dihydrogen phosphate [4]. Vijayalakshmi *et al* [5] reported dipotassium hydrogen orthophosphate, magnesium sulfate and ammonium sulfate as good phosphorus and sulfur sources for the growth of *A. ricini*. In the present study, phosphorus and sulfur sources like diammonium hydrogen orthophosohate, magnesium sulfate and ammonium sulfate supported good growth of *A. porri*.

Phytotoxicity of culture filtrates of *A. porri*

To study the phytotoxic effect of culture filtrate on seed germination, radicle and plumule elongation, the seeds were placed on blotting papers moistened with fungal culture filtrate. Blotters moistened with sterile distilled water served as controls. Germination as well as seedling growth (radicle and plumule elongation) were adversely effected up to 80% when the seeds were treated with the fungal culture filtrate.

Metabolites of *Alternaria* species were reported to be toxic to a variety of crop plants. Suemitsu *et al* [14] reported that porritoxin produced by *A. porri* had an inhibitory effect on the seedling growth of stone leek and lettuce. Metabolites of *A. porri* such as porritoxin sulfonic acid with an isoindoline skeleton and other isoindolines showed phytotoxic effect on stone leek and lettuce seedlings [15]. Toxic effects of metabolites obtained from *Alternaria* species were also reported [16].

Antibacterial activity of *A. porri*

The metabolites produced by *A. porri* were screened for antibacterial potential using seeded plate technique. The metabolites produced by *A. porri* adversely affected the growth of *B. subtilis*, *E. coli* and *P. aeruginosa* (Table 2). They had no effect on the growth of *P. fluorescens* and *X. citri*. The secondary metabolites produced by fungi were reported to inhibit the growth of bacteria [17-18].

In vitro* evaluation of fungicides against *A. porri

Data on the effect of fungicides affecting the growth of *A. porri* are recorded (Table 3). Out of the five fungicides tested, mancozeb was highly effective followed by blitox and benlate. A gradual reduction in fungal growth was found as the concentrations of the fungicides increased from 10 to 500 ppm. The efficacy of different fungicides like mancozeb and dicloran against the purple blotch disease was studied by Rahman *et al* [19] and mancozeb was reported as the best fungicide for the treatment of the disease [20-21].

Efficacy of different plant extracts against *A. porri*

Influence of plant extracts on the growth of *A. porri* was evaluated on the basis of its dry mycelial weights (Table 4). Growth of *A. porri* was restricted with all the plant extracts tested, but the degree of inhibition varied from 32 – 85%. Among the 15 plant extracts tested, *Mentha arvensis* was highly effective in checking the growth of *A. porri* followed by *Pongamia pinnata* and *Coriandrum sativum*. The inhibition level was high with increasing concentration of the plant extract.

Control of fungal pathogens with plant extracts was an effective and safe approach which is of eco-friendly nature. Several researchers [9, 22] also recommended the control of plant diseases

like purple blotch with plant extracts. Makelo *et al* [23] tested the efficacy of crude plant extracts of *Warburgia ugandensis*, *Solanum nigrum*, *Cleome gynandra* and *Acokanthera schimperi* on the growth of fungal pathogens including onion purple blotch agent, *A. porri* under *in vitro* conditions. They observed significant fungitoxicity with *S. nigrum* extract and fungistatic effect with *C. gynandra*.

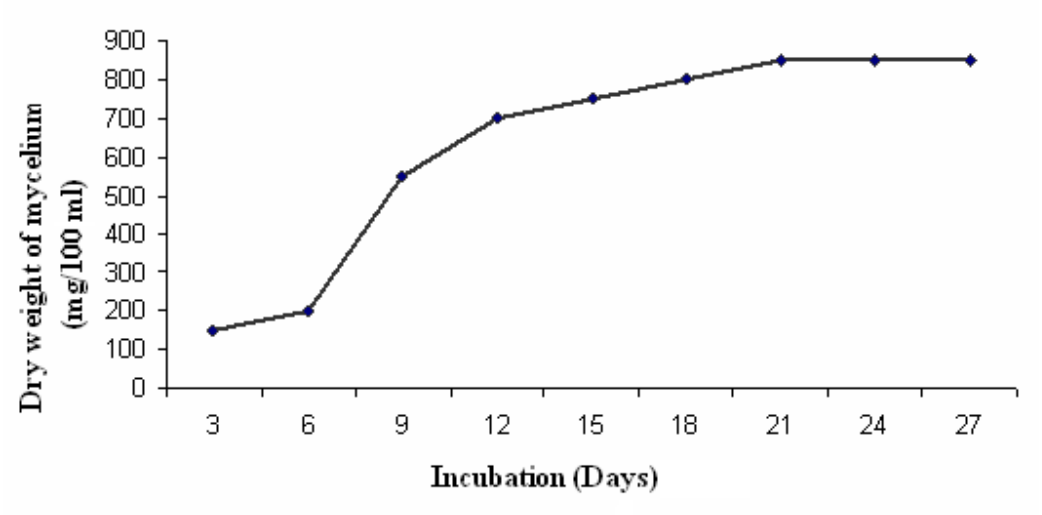

Fig.1: Growth pattern of *Alternaria porri* in Czapek-Dox broth

Table 1: Growth of *Alternaria porri* in Czapek-Dox broth supplemented with different nutrient sources

S. No	Nutrient sources	Dry weight of mycelium (mg/100 ml)
	Carbon sources	
1.	Dextrose	1010
2.	Galactose	1030
3.	Lactose	1120
4.	Maltose	920
5.	Xylulose	960
	Nitrogen sources	
6.	Ammonium nitrate	118
7.	Barium nitrate	243
8.	Magnesium nitrate	1023
9.	Potassium nitrate	563
10.	Urea	1220
	Phosphorus sources	
11.	Ammonium dihydrogen phosphate	1288
12.	Diammonium hydrogen orthophosphate	1345
13.	Disodium hydrogen orthophosphate	1187
14.	Sodium dihydrogen orthophosphate	976
15.	Zinc phosphate	1022
	Sulfur sources	
16.	Aluminum sulfate	656
17.	Manganous sulfate	1148
18.	Ammonium sulfate	1357
19.	Potassium sulfate	789
20.	Sodium sulphate	924

Table 2: Antibacterial activity of *Alternaria porri* against the test bacteria

Bacteria	**Area of the inhibition zone (mm^2)**
Bacillus megaterium	50
B.subtilis	240
Escherichia coli	235
Pseudomonas aeruginosa	120
P. fluorescens	---
Serratia marcescens	50
Xanthomonas citri	---

'---' No inhibition

Table 3: Effect of fungicides against *Alternaria porri in vitro*

Fungicides (ppm)	**Mycelial dry weight (mg/100 ml)**
Mancozeb	
10	145
50	127
100	102
500	93
Benlate	
10	162
50	134
100	121
500	111
Carbendazim	
10	223
50	207
100	194
500	161
Blitox	
10	157
50	124
100	112
500	101
Captafol	
10	241
50	214
100	196
500	151
Control (without any fungicide treatment)	1047

Table 4: Efficacy of plant extracts against *Alternaria porri*

S. No.	Name of the plant	Dry weight of mycelium in different concentrations of plant extracts (mg/100 ml)		
		10%	25%	50%
1.	*Achyranthes aspera*	563	542	482
2.	*Annona squamosa*	621	534	432
3.	*Azadirachta indica*	725	692	563
4.	*Calotropis gigantea*	602	542	436
5.	*Carica papaya*	654	562	467
6.	*Catharanthus roseus*	652	556	462
7.	*Coriandrum sativum*	422	396	353
8.	*Datura metel*	554	526	473
9.	*Mentha arvensis*	172	165	124
10.	*Murraya koenigii*	575	549	494
11.	*Ocimum sanctum*	636	521	412
12.	*Pongamia pinnata*	368	322	296
13.	*Psidium guajava*	730	696	575
14.	*Tagetes patula*	746	698	582
15.	*Tridax procumbens*	646	533	447
	Control (without any plant extract)	1100		

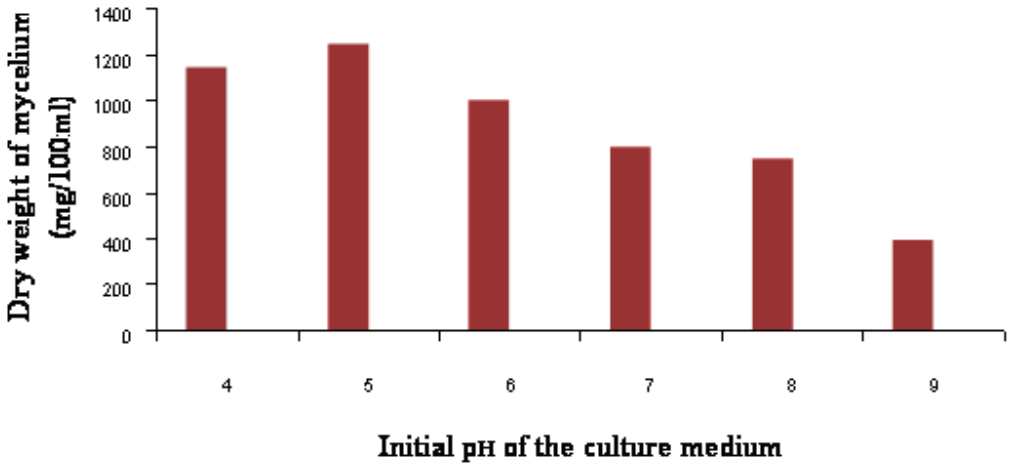

Fig. 2: Effect of initial pH on growth of *Alternaria porri*

CONCLUSION

In the present study, *A. porri*, the causal agent of purple blotch was isolated from the diseased leaves of onion. Cultural and nutritional parameters affecting the growth of pathogen were determined. The toxicity of the metabolites produced by the pathogen was tested and proved by using phytotoxicity and antibacterial activity as the test criteria. Besides, the study of the control of the pathogen by employing different fungicides showed mancozeb as the effective one for its growth inhibition and extracts of *Mentha arvensis* exhibited high efficacy to inhibit the growth of the pathogen among the plant extracts tested.

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