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## Phytotoxic effect of culture filtrate of *Alternaria alternata* on cucumber (*Cucumis sativus* L.) causing *Alternaria* leaf spot disease

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### Abstract

Cucumber (*Cucumis sativus* L.) is an important vegetable crop of Rajasthan as well as India and belongs to family Cucurbitaceae. *Alternaria* leaf spot disease of cucumber caused by *Alternaria alternata* is an important constraint to this crop in quality and monetary terms. A study was conducted to see the toxic effect of culture filtrate of *Alternaria alternata* on seedlings of three varieties (Cucumber Long, F-1 Hybrid Liberty and Summer Express-40) with four concentrations (25, 50, 75 and 100%) and 15, 30, 45 and 60 days old culture filtrate. Among concentrations, 100 per cent concentration of filtrate was highly phytotoxic as it gave minimum seed germination (56.25%) and seedling vigour index (7185.94) as compared to untreated check (92.50% and 18615.63, respectively) on Summer Express-40 variety. In age old filtrate study, 60 days old culture filtrate was more toxic in lowering seed germination (41.25%) and vigour index (3557.81) of variety F-1 Hybrid Liberty over untreated (86.75% and 16485.31). Conclusively, it is clear that released exudates of the pathogen in soil may be phytotoxic and can reduce seed germination and vigour index of plants that ultimately results in economic loss to the end users.

**Keywords:** Cucumber, *Alternaria* leaf spot, *Alternaria alternata*, culture filtrate, phytotoxicity

### Introduction

Cucumber (*Cucumis sativus* L.) is an important vegetable and one of the most popular members of the Cucurbitaceae family (Lower and Edwards, 1986)<sup>[5]</sup>. It is one of the quickest maturing vine vegetable crops and is the second most widely cultivated cucurbit after watermelon. It is a popular and widely cultivated summer vegetable in India. It is one of the important vegetable crops which supply edible product and fibres. Cucumbers are frost sensitive and are cultivated in the open or in greenhouses, depending on the type. Greenhouse cucumbers are parthenocarpic (produce fruit without fertilization of ovules), and the fruits are usually seedless. The immature fruits of cucumber are used as salad and for pickling. The fruit is also used as an astringent and antipyretic. The seed oil is used as antipyretic. Fruits are good for people suffering from constipation, jaundice and indigestion. In India, cucumber is commonly grown in Harayana, Karnataka, Madhya Pradesh, Andhra Pradesh and Rajasthan, generally towards the riversides. Rajasthan state provides the maximum potential for the production of cucumber because of its agro-climatic conditions are best suited for their growth and yield.

In India, cucumber production is 1142.03 thousand tonnes with an area of 78.16 thousand hectares and productivity (14.61 tonnes/ha) (Anonymous 2017). In Rajasthan, it is mainly cultivated in Jaipur, Alwar, Bharatpur, Ganganagar, Bhilwara, Sawai Madhopur and Dausa districts. In Rajasthan, cucumber production is 10.50 thousand tonnes with an area of 3.75 thousand hectares and productivity (2.8 tonnes/ha) (Anonymous 2017).

Among several diseases, *Alternaria* leaf spot disease of cucumber caused by *Alternaria alternata* is a serious concern (Zhou and Everts, 2008)<sup>[9]</sup> in the Mid-Atlantic United States and other parts of the world. Among symptoms, lesions tend to appear first on the older leaves as small circular spots and these are light brown with a light center and form concentric dark rings as they enlarge. Fruit infections begin as sunken brown spots and may later develop a dark powdery appearance as the fungus sporulates. Morphologically, fungus produces conidiophores arising singly or in small groups, pale to golden brown, up to 50 µm long, 3–6 µm thick, with one or more distinct conidial spores. Conidia are in branched chains of up to 15–20, sometimes separated by short secondary conidiophores, straight or slightly curved, obclavate, obpyriform, ovoid or ellipsoidal, with a short cylindrical beak, 7–25 µm long, 5–12

$\mu\text{m}$  wide, olivaceous, variously warted to verrucose, 1–7 (commonly 3) transverse septa, 0–2 longitudinal or oblique septa, constricted at septa. Hamid Reza Mirkarimi *et al.* (2013) conclude that leaflets received a 1000- $\mu\text{l}$  droplet of the *A. alternata* culture filtrate and were inoculated by spraying with a suspension of 105 conidia/ml of isolate *A. alternata* in the greenhouse method. *In vitro* selection of fungal isolates of *A. alternata*, chlorotic and necrotic symptoms began 1 to 2 days after inoculation, but the assessment of greenhouse symptoms appeared 6 - 10 days after inoculation. The area under the disease progress curve values were presented by analysis of variance (ANOVA), and they were compared using Duncan's test ( $\alpha = 0.01\%$ ). In both methods, there was a significant difference between the potato genotypes ( $P < 0.01$ ). For *In vitro* selection and evaluation greenhouse, Casmos were resistant to at least figure and Marfona genotype had the highest resistance. Srinath R and Ramgopal S. (2010) [7] that *In vitro* plantlets were obtained from the selected callus on Murashige and Skoog's (MS) medium fortified with 2.0 mg/L N-6-Benzyl amino purine (BAP) and 0.5 mg/L Naphthalene acetic acid (NAA). The plantlets obtained were rooted on MS medium fortified with 1.0 mg/L NAA or IBA, NAA proved better for rooting than IBA. In the preliminary experiments, *in vitro* developed plants from selected callus showed fewer symptoms of disease when spore suspensions of *A. helianthin*, was sprayed on the leaves.

## Materials and Methods

### Preparation of culture filtrate

The pathogen *Alternaria alternata* was isolated aseptically from diseased leaves of cucumber from the field and multiplied on Potato Dextrose Agar (PDA) for further studies. Small bit (5 mm dia) from seven days old culture of pathogen was inoculated in 250 ml conical flasks having 50 ml of Potato Dextrose Broth (PDB) and incubated at  $25 \pm 1$  °C for 15, 30, 45 and 60 days in BOD incubator. Fungal mycelium mat was separated by passing fungal culture through muslin cloth and then sterilized through Whatman No. 1 filter paper. The culture filtrate was further sterilized by passing through millipore filter of 0.22  $\mu\text{m}$  size and heated at 100 °C for 2 minutes to inactivate enzymes. Autoclaved flasks containing Potato Dextrose Broth without pathogen were kept as control (Watpade and Mehta, 2013) [8].

### Effect of different concentrations of culture filtrate on seed germination and seedling vigour of cucumber varieties (*in vitro*)

Culture filtrate and sterilized water were used to make 25%, 50%, 75% and 100% of culture filtrate solution. Seeds of different cucumber varieties namely Summer Express-40, Cucumber Long, F1 Hy. Liberty. were surface sterilized with sodium hypochlorite (1%) and 20 seeds were placed in sterilized three blotter paper containing Petri plates, each poured with 10 ml of culture filtrate except control and incubated in BOD chamber at  $25 \pm 1$  °C with 80% relative humidity. Percentage of seed germination and radicle length and plumule length recorded after 14 days.

**Effect of different ages of culture filtrate on seed germination and seedling vigour of cucumber varieties (*in vitro*):** Prepared different days old culture filtrate *viz*, 15, 30, 45 and 60 days as above. Seeds of different cucumber varieties namely, Summer Express-40, Cucumber Long, F1

Hy. Liberty, were surface sterilized with sodium hypochlorite (1%) and 20 seeds were placed in sterilized four blotter paper containing Petri plate, each poured with 10 ml of culture filtrate except control and incubated in BOD chamber at  $25 \pm 1$  °C with 80% relative humidity. Percentage of seed germination and radicle length and plumule length were recorded at 14 days of incubation. The experimental design was completely randomized (CRD) with four replications.

**Germination percentage:** The number of seedling emerged were counted seven days after incubation in each of accession tested and the mean was expressed in percentage. Germination percentage was calculated as below:

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds incubated}} \times 100$$

**Root and Shoot length** was measured after fourteen days of incubation.

**Plumule length:** Shoot length was measured from the collar region to the top in each of accession tested and the mean value was expressed in millimeters.

**Radicle length:** Root length was measured from the collar region to tip of the primary root in each of accession tested and the mean value was expressed in millimeters (mm).

**Seedling vigour index:** Seedling vigour was also calculated by following formula of Abdul-Baki and Anderson (1973) [1]. Seedling Vigour Index = Germination % x (Radicle length + Plumule length)

## Results and Discussion

### Effect of different concentrations of culture filtrate on seed germination and seedling vigour of cucumber varieties (*in vitro*)

The effect of culture filtrate concentrations of *Alternaria alternata* were observed on seed germination, seedling growth (radicle and plumule length) and vigour index (Table 1). The culture filtrate of pathogen inhibited the seed germination and seedling growth (radicle and plumule length) and vigour index. The inhibitory effect of culture filtrate was observed very low at 25% concentration. At 100% concentration the germination, seedling growth (radicle and plumule length) and vigour index were found least. The results showed that cucumber variety Summer Express-40 exhibited highest and F1 Hy. Liberty showed least germination per cent, seedling growth (radicle and plumule length) and vigour index at all concentrations. Maximum per cent reduction of germination per cent also found with 100 per cent concentration to all varieties. According to result increase the concentration of culture filtrate show adverse effect on seed germination, seedling growth and vigour index. Similar work done by Rani and Aggarwal (1999) [6] *B. juncea* [Indian mustard] seeds were soaked for 24 h in 20%, 40%, 60%, 80% and 100% solutions of culture filtrates of *Alternaria alternata*, *Aspergillus flavus*, *Helminthosporium sativum* [*Cochliobolus sativus*] and *Curvularia lunata* [*Cochliobolus lunatus*]. All culture filtrates decreased seed germination with increase the concentrations. *Alternaria alternata* filtrate had the greatest effect on germination ranging from 10-60% with the different concentrations.

**Table 1:** Effect of different concentrations of culture filtrate of *A. alternata* on seed germination and seedling vigour of cucumber varieties (*in vitro*)

Cucumber Varieties	Different Concentrations of culture filtrate*																			
	0%				25%				50%				75%				100%			
	Germination (%)	Plumule length (mm)	Radicle length (mm)	Vigour index	Germination (%)	Plumule length (mm)	Radicle length (mm)	Vigour index	Germination (%)	Plumule length (mm)	Radicle length (mm)	Vigour index	Germination (%)	Plumule length (mm)	Radicle length (mm)	Vigour index	Germination (%)	Plumule length (mm)	Radicle length (mm)	Vigour index
Cucumber Long	91.25 (72.79)	93.50	104.00	18021.88	72.50 (58.37)	75.75	91.50	12125.63	68.75 (56.01)	71.75	84.25	10725.00	55.00 (47.87)	66.50	62.75	7108.75	47.50 (43.57)	58.50	56.00	5438.75
F1 Hy. Liberty	88.75 (70.40)	87.25	98.50	16485.31	68.75 (56.01)	73.75	77.25	10381.25	61.25 (51.50)	66.50	70.25	8375.94	53.75 (47.15)	61.25	58.25	6423.13	43.75 (41.41)	44.50	53.00	4265.63
Summer Express-40	92.50 (74.11)	96.25	105.00	18615.63	80.00 (63.43)	80.75	102.50	14660.00	76.25 (60.83)	77.75	99.50	13515.31	67.50 (55.24)	68.75	90.50	10749.38	56.25 (48.59)	58.75	69.00	7185.94
S.Em+	2.82	2.95	3.21		2.46	2.47	3.19		2.36	2.39	3.12		2.10	2.11	2.88		1.75	1.83	2.15	
CD (P=0.05)	8.69	9.08	9.88		7.59	7.62	9.83		7.27	7.37	9.62		6.46	6.49	8.88		5.39	5.63	6.63	

Average of four replications

Figures given in parentheses are angular transformed value

**Table 2:** Effect of different ages of culture filtrate of *A. alternata* on seed germination and seedling vigour of cucumber varieties (*in vitro*)

Cucumber Varieties	Different ages of culture filtrate*																			
	Control				15 Days				30 Days				45 Days				60 Days			
	Germination (%)	Plumule length (mm)	Radicle length (mm)	Vigour index	Germination (%)	Plumule length (mm)	Radicle length (mm)	Vigour index	Germination (%)	Plumule length (mm)	Radicle length (mm)	Vigour index	Germination (%)	Plumule length (mm)	Radicle length (mm)	Vigour index	Germination (%)	Plumule length (mm)	Radicle length (mm)	Vigour index
Cucumber Long	91.25 (72.79)	93.50	104.00	18021.88	68.75 (56.01)	73.00	88.50	11103.13	66.25 (54.48)	67.50	81.25	9854.69	58.75 (50.04)	62.00	61.00	7226.25	43.75 (41.41)	46.00	50.00	4200.00
F1 Hy. Liberty	88.75 (70.40)	87.25	98.50	16485.31	65.00 (53.73)	71.50	74.75	9506.25	61.25 (51.50)	65.25	69.25	8238.13	53.75 (47.15)	52.75	56.25	5858.75	41.25 (39.96)	40.75	45.50	3557.81
Summer Express-40	92.50 (74.11)	96.25	105.00	18615.63	80.00 (63.43)	76.50	98.25	13980.00	73.75 (59.18)	73.50	97.50	12611.25	60.00 (50.77)	64.00	90.50	9270.00	46.25 (42.85)	56.75	66.25	5688.75
S.Em+	2.82	2.95	3.21		2.48	2.34	3.06		2.28	2.26	3.06		1.84	1.97	2.90		1.42	1.78	2.09	
CD (P=0.05)	8.69	9.08	9.88		7.63	7.20	9.42		7.01	6.95	9.42		5.66	6.08	8.92		4.37	5.48	6.44	

Average of four replications

Figures given in parentheses are angular transformed value

### Effect of different ages of culture filtrate on seed germination and seedling vigour of cucumber varieties (*In vitro*)

It is clear from the results that toxicity of culture filtrate increases with increase in age (Table 2). The results showed that Minimum germination per cent, seedling growth (radicle and plumule length) and vigour index found with 60 days old culture filtrate to all varieties and cucumber variety Summer Express-40 exhibited highest and F1 Hy. Liberty showed least germination per cent, seedling growth (radicle and plumule length) and vigour index among the varieties. Maximum per cent reduction of germination per cent also found with 60 days old culture filtrate to all varieties. *Alternaria* produces a group of mycotoxins as toxin, alternariols, altertoxins etc. Host specific toxins of *Alternaria* sp. play an important role in pathogenesis. While many species of *Alternaria* produce toxins with rather broad host ranges, a closely-related group of agriculturally important *Alternaria* species produce selective toxins with a very narrow range often to the cultivar level (Holliday, 1980). In the present investigation, the fungal culture filtrate was prepared on Potato Dextrose Broth. Different days old culture filtrate viz., 15, 30, 45 and 60 days, tested on cucumber varieties. Sixty days old culture filtrate were the most inhibitory effect on seed germination, seedling growth and vigour index. Summer express-40 variety showed highest germination, seedling growth and vigour index. Whereas, F1 Hy. Liberty showed least germination, seedling growth and vigour index. Bavaji et al. (2000) [2] also observed that old ages culture filtrates effects were the most inhibitory to seed germination and root and shoot elongation. He tested 7, 15, 30, 45 and 60-day-old culture filtrate on seed germination and seedling growth of *Sesamum indicum* and results indicated that 60-day-old culture filtrates effects were the most inhibitory to seed germination and root and shoot elongation.

Conclusively, it is clear that released exudates of the pathogen in soil may be phytotoxic and can reduce seed germination and vigour index of plants that ultimately results in economic loss to the end users.

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