Effect of pH, temperature and light on the growth of selected fungal strain *Alternaria alternata* (FCWH#46) for the management of water hyacinth (*Eichhornia crassipes*)

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Abstract - *Alternaria alternata* is a pathogen of the water hyacinth (*Eichhornia crassipes*), which is an important highly invasive aquatic weed. We conducted a survey of this pathogen at different sites in the Jabalpur region. The extremely high and very low temperature decreases the growth of fungi. To determine the growth rate of fungi, fungal isolates were grown in-vitro on agar. Effect of different pH levels, temperature, and light intensity were tested against the growth of *Alternaria alternata* (FCWH#46) under *in vitro* conditions. The results of experiment indicated that the maximum Phytotoxic effect (in %) in pH range of 5.0 and maximum Phytotoxic effect (in %) in temperature 25° range. The test fungus was grown under three different light regimes viz., 24 hours light, 24 hours darkness and 12 hours of alternate dark and light period.

Key words: *Alternaria alternata* (FCWH#46), pH, light, temperature, water hyacinth.

1. Introduction

Water hyacinth is a free-floating perennial aquatic plant (or hydrophytes) native to tropical and sub-tropical South America. Originally these weeds were introduced as ornamental. Water hyacinth best grows in slow moving water bodies, lakes, streams, ponds, waterways, ditches, and backwater areas. It can survive frosting but requires temperatures above 10 degrees Celsius for growth, with optimal growth occurring between 28°C and 30°C but not exceeding 35 °C. It tolerates a pH range from 4.0 to 8.0 and can survive in water with salinities up to 15 per cent that of sea water. One of the fastest growing plants known, water hyacinth reproduces primarily by way of runners or stolons, which eventually form daughter plants. Each plant can produce thousands of seeds each year, and these seeds can remain viable for more than 28 years (Sullivan, Paul and Wood, Rod. 2012). The common water hyacinth (*Eichhornia crassipes*) is vigorous growers known to double their population in two weeks.

Water hyacinth mats clog waterways, making boating, fishing, swimming, and almost all other water activities, impossible. These plants cover the water's surface in a mat-like sheet and restrict sunlight that underwater native plants need for growth (Villamagna, 2010). Eventually this underwater vegetation dies and decays depleting dissolved oxygen in the water, which is needed for underwater life. As well as depleting oxygen, choking waterways and stifling recreation, water hyacinth provides prime habitat for disease vectors such as mosquitoes and parasitic flatworms.

Alternaria alternata (Fr.) Keissler is a cosmopolitan fungus. This facultative pathogen has been isolated from diseased Water hyacinth worldwide. (Ellis, 1971; Domsch *et al*., 1980; Farr *et al*., 1989; EL-Morsy, 1999, 2000; EL-Morsy *et al*., 2000) .The fungus induces disease symptoms (spots and lesions) mainly on leaves and less severely on stolons and finally leads to complete death of the plant. Hydrogen ion concentration has important effect in the enzymatic control system of fungi. It influences the enzymatic action by modifying surface reaction and permeability, by facilitating or preventing the entry of various substances like vitamins, organic acids and minerals into the fungal cell. pH,temperature and light are the most important physical factor that affects the cellular activities of fungi both in vivo and *in vitro*.

In this research paper we are studies that effect of pH and Temperature and light in the growth of selected fungal strain *Alternaria alternata* (FCWH#46) for the management of water hyacinth because pH, Temperature and light are most important parameter for growth and sporulation

of fungal strain for large scale production.

2. MATERIAL AND METHODS

2.1 Fungal culture

The samples brought from the field were further analysed for the isolation of fungi for the isolation of fungi. For quantitative estimation and comparing the fungal flora of different disease samples "Pour plate" method was followed (Walksman,1922). Then culture was stored at 4°C for further study.

2.2 Fungal growth at various temperatures

To study phytotoxin production and effect of different temperatures mycelial growth and sporulation of test pathogen was observed different temperatures (viz., 0, 5°C, 10°C, 15°C, 20°c, 25°C,28°C,30°C,35°C and incubated for 14 days. Mycelia biomass was recorded as described earlier.

2.3 Fungal growth at various hydrogen ion concentrations

To determine best suited pH for phytotoxin production by the test strain, it was grown at different pH levels i.e. 3, 4, 5, 6, 7 and 8. The pH of the basal medium was adjusted by addition of 1N HCl or NaOH before autoclaving and Systronic pH meter was used for pH measurements. The pH exhibiting highest phytotoxin production in terms of phytotoxic activity was selected as optimum pH for further studies.

2.4 Effect of light: For studying the effect of light, inoculated Petri-plates were exposed to four light conditions *viz.,* 12 hours of continuous light and 12 hours of continuous darkness, 24 hours of continuous darkness and 24 hours of continuous light. Light was provided with the help of white fluorescent tube light (four Phillips TL 40 W /33, 1000 lux) and continuous darkness was maintained by wrapping the Petri-plates with black paper. For *alternata* light and darkness, plates were kept in normal condition.

3. RESULTS AND DISCUSSION: In this study determined that among the local pathogens isolated *Alternaria alternata* (FCWH#46) caused maximum damage to water hyacinth. Lily and Barnett, 1951 reported that physiological specificity of organism extends to the configuration as well as composition of the molecule.

3.1 Effect of different pH levels on growth and sporulation of *Alternaria alternata* (FCWH#46)

The mycelial growth of the fungus initiated immediately within 24 hours of inoculation at pH 4.5 and 5 followed by pH 4, 3.5, 6 and 7. At pH 8 and 9 initiation of mycelial growth appeared only after 48 and 72 hours of incubation respectively. It is illustrated in Table (i) that by the 7^{th} day maximum radial growth was observed at pH 5. It was closely followed by pH 4.5, 4 and 3.5. The colony diameter of the pH 4, 4.5 and 3.5 were statistically at par with each other. Similarly maximum sporulation was observed at pH 5. No sporulation was seen at pH 8 and 9. (Shabana *et al.,* 2000) reported that in *Alternaria eichhorniae,* the best mycelial growth was obtained at pH 7 and least at pH 4. The rate of mycelial growth increased as pH increased from 4 to 7 and then decreased from pH 7 to pH 9.7, while the best sporulation was obtained at pH 5, 5.6 and 9.7.

3.2 Effect of different pH levels on biomass and toxin production by *Alternaria alternata* (FCWH#46)

The results presented in Table (ii) and (iii) that the fungi preferred to grow well at pH range 3.5 to 5 indicating that the pathogen cannot grow well in alkaline medium. The pH preferred for high toxin production was 5 as it showed the best toxicity to water hyacinth. At the end of 21 days it was seen that the pH of the culture filtrate changed with the growth of the fungi. The lower levels of pH (3.5 - 6.0) increased after the growth of the fungi where as reverse was true for higher levels of pH (8-9). The pH level of 7.0 however remained unchanged after the growth of the fungi. Change in pH of the medium may be attributed to the formation of secondary metabolites or hydrolytic products due to active growth of the fungi (Lilly and

Barnett, 1951., Griffin, 1981) started fungi often change the pH of the culture medium drastically during growth and such radical changes in pH are difficult to control.

3.3 Effect of temperature on growth and sporulation of *Alternaria alternata* (FCWH#46)

It is evident from the Table (iv) that test fungi could grow within a wide range of temperature i.e., 10°C to 35°C. Maximum growth was recorded at 25°C. Beyond this the growth gradually decreased with increase or decrease in temperature. 5°C failed to support any growth. Comparable trend was recorded in case of sporulation, which was more or less high on the temperatures ranging from 20°C to 30°C. Excellent sporulation was obtained at temperatures 25° and 30°C followed by 20°, 35°, 15° and 10°C. Zhang *et al*., 2001reported that the optimal temperature for the mycelial growth of *Plectosporium tabacinum* was between 22 and 25°C, but the optimal temperature for spore production was at either 20 or 30°C, depending upon the nutrient medium.

3.4 Effect temperature on biomass and toxin production by *Alternaria alternata* (FCWH#46)

Results shown in Table(v) illustrates maximum mycelial biomass was obtained at temperature 25°C followed by 30°, 35° and 20°C. At 15° and 10°C low mycelial growth was obtained while at 5°C no growth took place. It was further seen that good growth induced higher toxin production and pH drifted towards neutrality. Excellent phytotoxin production was seen at temperature range of 25° to 35°C with maximum at 30°C. Similar differences in temperature requirement for mycelial growth and phytotoxin production has been shown by a number of workers. Hasan ,1996 reported that optimum temperature for toxin production by *A. alternata* was 28°C for Alternariol and Allernariol monomethyl ester, 21°C for Tenuazonic acid and 14°C for Allertoxin I and II. Demain, 1972 reported that Ashbya gossypi induced over production of riboflavin optimal at 28°C although the optimal growth was around 37°C.

3.5 Effect of light on growth and sporulation of *Alternaria alternata* (FCWH#46)

According to data presented in Table (vi) and vii mycelial growth of *A. alternata* (FCWH#46) was seen maximum when 24 hours of continuous darkness was provided. Culture plates kept under alternata 12 hours of light and darkness followed it. Lowest growth was seen in plates kept in 24 hours of light. Radial bands of dark and light shade could be seen after 3 days of incubation in plates that were provided with light and darkness of 12. (Sharma *et al*., 2005) reported that mycelial growth of *Fusarium oxysprium* f. sp. lini was maximum when alternata light and darkness of 12 hours was provided. It was followed by 24 hours of continuous light while radial growth under complete darkness was lowest.

Maximum sporulation was obtained under the condition of complete darkness. It was followed by alternata light and darkness of 12 hours. Scarce sporulation was seen when 24 hours of continuous light was provided. Contrary to this (Cotty and Mishagi, 1985) observed that diurnal light regime is required for *A. tagetica* to sporulate similarly Sharma *et al*., 2005 reported excellent, good and fair sporulation in *Fusarium oxysprium* f. sp. lini. under alternata light and darkness of 12 hours, 24 hours of continuous light and 24 hours of continuous darkness respectively.

3.6 Effect of light on biomass and toxin production by *Alternaria alternata* (FCWH#46)

Light exerts an important effect upon cellular metabolites through changes in chemical environment. Data presented in the Table (viii) clearly indicate that *A. alternata* (FCWH#46) could grow well under all four light regimes. Maximum mycelial dry weight was obtained under condition of total darkness of 24 hours followed by alternata light and darkness of 12 hours each and least on 24 hours light and darkness.

Maximum phytotoxin production was observed at light regime of alternata light and darkness of 12 hours each followed by 24 hours of darkness and least on 24 hours of light (Table ix).

Table No. (i) Effect of hydrogen ion concentration on growth and sporulation of *Alternaria alternata* (FCWH#46)

S. No.	pH	COLONY DIAMETER (MM) (MEAN ± SD)			SPORULATION
		3 DAYS	5 DAYS	7 DAYS	
1	3.5	29.9 ± 0.44	42.8 ± 0.47	58.8 ± 0.28	2.9×10^6
2	4.0	30.1 ± 1.24	45.8 ± 0.72	57.8 ± 1.1	2.7×10^6
3	4.5	33.2 ± 0.42	46.4 ± 0.41	63.7 ± 0.57	3.3×10^6
4	5.0	34.6 ± 0.34	47.1 ± 0.88	67.5 ± 0.60	3.2×10^6
5	6.0	29.4 ± 0.88	35.9 ± 0.22	56.3 ± 1.07	1.9×10^6
6	7.0	27.6 ± 0.72	36.2 ± 0.43	49.0 ± 1.74	1.6×10^6
7	8.0	23.3 ± 0.16	29.5 ± 0.41	41.1 ± 0.27	0
8	9.0	21.6 ± 1.10	27.7 ± 0.17	35.1 ± 0.47	0

- Culture medium used - Richard's broth
- Incubation at 25 ± 1°C

Table No. (ii) Effect of hydrogen ion concentration on biomass production of *Alternaria alternata* (FCWH#46)

S.No.	pH	Change in pH in the broth on 21st day	MYCELIAL DRY WEIGHT AFTER 21 DAYS (in gm/l) (MEAN ± SD)
1	3.5	8.3	8.2 ± 0.5
2	4.0	7.9	11.2 ± 0.6
3	4.5	7.3	12.2 ± 0.4
4	5.0	7.1	14.4 ± 1.2
5	6.0	8.7	6.3 ± 1.1
6	7.0	7.1	4.8 ± 0.7
7	8.0	7.1	3.1 ± 0.7
8	9.0	8.4	2.7 ± 0.8

- Culture medium used - Richard's broth
- Values are means ± SD of three observation
- Incubation at 25 ± 1°C

Table No. (iii) Phytotoxic effect of culture filtrate on *Alternaria alternata* (FCWH#46) grown at different pH levels

S.No.	pH	Phytotoxic effect (in %)		
		1 day	3 days	7 days
1.	3.5	46.8	73.1	82.1
2.	4.0	57.1	76.0	83.7
3.	4.5	67.4	79.2	99.8
4.	5.0	71.4	85.4	100.0
5.	6.0	18.6	45.6	60.2
6.	7.0	24.6	37.2	54.8
7.	8.0	5.1	8.3	11.2
8.	9.0	4.9	8.2	14.3

Table No. iv Effect of temperature on growth and sporulation of *Alternaria alternata* (FCWH#46)

TEMP. (IN°C)	COLONY DIAMETER (MM) (MEAN±SD)			SPORULATION
	3 DAYS	5 DAYS	7 DAYS	
5°	0.0	0.0	0.0	0.0×10^6
10°	7.2 ± 1.21	15.9 ± 1.4	19.8 ± 0.42	1.3×10^6
15°	12.7 ± 0.43	24.2 ± 0.42	33.1 ± 0.56	1.4×10^6
20°	20.1 ± 0.72	28.9 ± 0.33	39.4 ± 1.13	2.6×10^6
25°	28.7 ± 0.43	41.2 ± 0.37	51.7 ± 0.4	3.2×10^6
30°	22.7 ± 0.41	41.7 ± 0.74	52.8 ± 0.29	3.3×10^6
35°	22.3 ± 0.98	33.4 ± 0.76	42.1 ± 0.47	2.3×10^6

(Culture Media Used – Richard's broth)

Table No. (v) Effect of temperature on biomass production of *Alternaria alternata* (FCWH#46)

TEMPERATURE (IN °C)	Change in pH in broth on 21st day	MYCELIAL DRY WEIGHT AFTER 21 DAYS (in gm/l) (MEAN± SD)
5°	5.2	0.0 ± 0.0
10°	5.1	2.2 ± 1.5
15°	6.7	5.9 ± 1.2
20°	4.8	9.9 ± 1.1
25°	5.4	13.5 ± 1.1
30°	7.2	12.2 ± 3.5
35°	7.5	12.1 ± 4.1

- Culture medium used - Richard's broth
- Values are means ± SD of three observation

Table No. vi Phytotoxic effect of culture filtrate of *Alternaria alternata* (FCWH#46) grown at different temperatures

TEMPERATURE (IN °C)	Phytotoxic effect (in %)		
	1 day	3 days	7 days
5°	0.0	0.0	0.0
10°	0.0	0.0	0.0
15°	0.0	0.0	7.2
20°	13.5	34.6	67.8
25°	70.7	77.3	99.3
30°	66.0	76.0	98.7
35°	65	75.3	97.7

(Culture medium used - Richard's broth)

Table No. (vii) Effect of light on growth and sporulation of *Alternaria alternata* (FCWH#46)

Diurnal light period (hrs)	COLONY DIAMETER (mm)			SPORULATION
	3 DAYS	5 DAYS	7 DAYS	
24	28.3	4.7	56.8	2.2×10^6
0	34.2	52.8	71.6	3.6×10^6
12	27.5	44.5	64.5	2.8×10^6

(Incubation at 25± 1°C, pH = 5)

Table No. (viii)Effect of light on biomass production of *Alternaria alternata* (FCWH#46)

Diurnal light Period (hrs)	CHANGE IN pH IN BROTH ON 21st DAY	MYCELIAL DRY WEIGHT AFTER 21 DAYS (in 2 m/l)
24	7.1	9.64
0	6.2	13.7
12	5.8	10.1

- Culture medium used - Richard's broth
- Incubation at 25± 1°C, pH = 5
- Light was provided with the help of white fluorescent tube light (four Phillips TL 40 W/33, 1000 lux)

Table No. (ix) Phytotoxic effect of *Alternaria alternata* (FCWH#46) grown under different photoperiodic condition on water hyacinth

S. No	Diurnal light period (hrs)	PHYTOTOXIC EFFECT (in%)		
		1 day	3 days	7 days
1.	24	24.8	38.6	57.0
2.	0	45.3	68.6	90.2
3.	12	61.8	76.1	98.2

4. CONCLUSION From the above result, it can be concluded for this various environmental conditions like pH, light, and temperature requirements for growth, sporulation and toxin production of *Alternaria alternata* (FCWH#46) were studied. This study suggests that fungal species have the potential to control water hyacinth biologically and provides baseline data for biological control efforts in the future.

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