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Screening and Media Optimization of *Alternaria petroselini* for Bioherbicide Production Against *Eichhornia crassipes*

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Abstract

Water hyacinth (*Eichhornia crassipes*) is one of the world's most destructive aquatic weeds, causing severe ecological and economic losses by reducing dissolved oxygen, deteriorating water quality, and interfering with agricultural productivity. The present investigation aimed to optimize culture media for enhanced growth, biomass accumulation, and phytotoxic metabolite production of *Alternaria petroselini* (FCEC#38) for potential bioherbicidal application. The fungus was cultured on various natural, semi-synthetic, and synthetic media. Growth parameters including radial growth, sporulation, biomass production, pH changes, and phytotoxic activity of cell-free culture filtrates were evaluated under controlled laboratory conditions. Among the tested media, Potato Dextrose Agar and Potato Dextrose Broth supported maximum radial growth (72.1 mm at 7 days), sporulation (3.1×10^6 spores/mL), and mycelial dry weight (13.4 g/L). Culture filtrates obtained from Potato Dextrose Broth and Richard's broth exhibited the highest phytotoxicity against water hyacinth (100% injury after 7 days). Statistical analysis (ANOVA, $p \leq 0.05$) confirmed significant differences among media treatments. The findings demonstrate that nutrient composition plays a critical role in biomass and toxin production, and optimized media can enhance large-scale bioherbicide production for sustainable management of water hyacinth.

Keywords: *Eichhornia crassipes*, *Alternaria petroselini*, bioherbicide, media optimization, phytotoxicity

Introduction:

Weeds are unwanted and undesirable plants, which interfere with the utilization of land and water resources and thus adversely affect human welfare. *Eichharnia crassipes* Commonly known as water hyacinth is a free-floating aquatic weed. It was introduced into many countries during the late 19th and early 20th century as an ornamental plant but soon became a menace. It is known as the worst aquatic weed in the world because of its unique growth habit, physiological traits, and reproductive strategies that enable it to grow and spread quickly in freshwater environments, doubling its biomass in roughly 6 to 18 days (Holm et al., 1977; Gopal, 1987; Center, 1994). The most ignored aspect of the harm done by water hyacinth is its direct impact on fresh water itself. The plant cover provides obnoxious smell, suspended particulate matter in water. It also causes oxygen depletion load on the water body. This action lowers the natural ability of water body to absorb organic pollution and creates unhygienic and odorous condition. It poses a grave threat to native habitats and seriously depletes water bodies of oxygen. Water hyacinth causes significant economic losses of up to 24 million dollars in rice fields by interfering with seed germination and seedling establishment (Mathur et al., 2005).

Although the control of weed using bioherbicides is attractive research and commercialization of these products are yet low. Since the first reports of using bioherbicides from colletotricum gloeosporium (Tebeest et al., 1992), at least 11 products were commercialized (Bailey et al.,2011, Ash, 2010). The research for biocontrol agents of weeds is increasing. Among the microorganisms that can be used, the fungal of *Alternaria alternate* were reported as potential agent. Gupta et al. (2022) evaluated the action of Alternaria sp. as bioherbicide. In the present study to investigate the effect of different media growth and toxic production of *Alternaria petroselini* for synthesis of bio-herbicide.

Material & Methods:

In the first stage of screening, 16 fungal pathogens were isolated, out of which four of the most virulent isolates were selected for further study. These included Alternaria petroselini (FCEC#38), Alternaria sp. (FCEC#25), Fusarium oxysporum (FCEC#2), and Rhizoctonia solani (FCEC#20). These isolates were screened again as described earlier, and Alternaria petroselini (FCEC#38) was found to be the most pathogenic among them.

Alternaria petroselini (FCEC#38) was isolated from diseased samples of water hyacinth collected from the Raipur Division. The fungus was isolated on PDA medium using the serial dilution and pour plate techniques. Based on its high pathogenicity, Alternaria petroselini (FCEC#38) was selected as a potential biocontrol agent. Further studies were conducted to screen suitable culture media for the synthesis of a bio-herbicide.

Preparation of different culture media:

Different solid and liquid media were prepared and tested to study the growth, sporulation and toxin production of *Alternaria petroselini* (FCEC#38).

- **Natural media** (Water hyacinth leaf extract)
- **Semi synthetic media** (Potato dextrose media, Martin's media, Sabouraud's media, Maltose Peptone, Yeast Glucose, Malt Extract)
- **Synthetic media (**Czapek's Media, Miller's Media, Mayer's Media, Lanolin Media, Richard's Media, Asthana & Hawker Media, Cohn's Media**.)**

Preparation of Cell Free Culture Filtrate:

500 ml Erlenmeyer flasks with 250 ml of pre-sterilized all selected media (Natural media, Semi synthetic media and Synthetic Media) were seeded with 5 mm discs from a robustly developing culture that was 10 days old on PDA medium at 28±1° in a Cana BOD incubator (Remi, India). The discs were separated by a sterilized

cork borer. For different days, (as per required) inoculated flasks were incubated in a BOD incubator.

Extraction of Cell Free Culture Filtrate:

The metabolized broth was centrifuged at 4000 x g for 15 to 20 minutes while being aseptically filtered using a preweighed Whatman filter paper no. 1 (Vikrant et al., 2006). To obtain crude culture filtrate, the pellet was discarded and the supernatant was re-filtered in vacuo using sterile 0.45 µm Minisart microfilters (Sartorius, Gottingen, Germany) (Walker and Templeton, 1978).

Biomass estimation in Liquid Media:

In order to encourage fungal development, cultures were incubated at 28±1°C. At the end of the incubation period, the contents of each 15 ml Erlenmeyer flask were filtered using Whatman No. 1 filter paper that had been previously dried and weighed. The filter sheets were dried in a hot air oven (Remi, India) for 24 to 36 hours at 90±1°C in order to attain a constant dry weight. They were then cooled in a vacuum desiccator (Chung and Tzeng, 2004). Each experiment was carried out in triplicate, and statistical validation was performed on the results.

Materials and Methods

Isolation and Selection of Pathogen

Sixteen fungal isolates were initially screened from diseased samples of water hyacinth collected from the Raipur Division. Four virulent isolates were selected for further evaluation: *Alternaria petroselini* (FCEC#38), *Alternaria* sp. (FCEC#25), *Fusarium oxysporum* (FCEC#2), and *Rhizoctonia solani* (FCEC#20). Based on pathogenicity assays, *A. petroselini* (FCEC#38) was identified as the most effective and selected for media optimization studies.

Culture Media

The following media were evaluated:

Natural medium: Water hyacinth leaf extract
Semi-synthetic media: Potato Dextrose Agar/Broth (PDA/PDB), Martin's, Sabouraud's, Malt Extract, Maltose Peptone, Yeast Glucose
Synthetic media: Czapek's, Miller's, Mayer's, Richard's, Asthana & Hawker's, Cohn's

Biomass Estimation

Flasks containing 250 mL sterilized broth were inoculated with 5 mm mycelial discs and incubated at 28 ± 1°C for 21 days. Mycelial mats were filtered through pre-weighed Whatman No. 1 filter paper and dried at 90 ± 1°C until constant weight. Dry weight was expressed as g/L. Experiments were conducted in triplicate.

Preparation of Cell-Free Culture Filtrate

Fermented broth was centrifuged at 4000 × g for 20 minutes and filtered through 0.45 µm membrane filters to obtain sterile culture filtrate.

Phytotoxicity Assay

Water hyacinth plants were sprayed with culture filtrates. Injury percentage was recorded after 1, 3, and 7 days using the formula:

Phytotoxicity (%) = (Injured leaf area / Total leaf area) × 100

Distilled water served as control.

Statistical Analysis

All experiments were conducted in triplicate. Data were analyzed using one-way ANOVA, and means were compared at $p \leq 0.05$.

Results

Effect of nutritional media on growth and sporulation of *Alternaria petroselini* (FCEC#38)

The results displayed in the graph 01 demonstrate that the morphology of *Alternaria petroselini* (FCEC#38) cultivated on various media noticeably. PDA showed the highest radial development, which was comparable to that of yeast glucose and Martin's media. Mayer's, Richard's, Miller's, Sabouraud's, Czapek's, waterhyacinth (hostleaf) extract, Richard's, Lenolin's, Asthana and Hawker's, and maltose peptone were the next in line. The sporulation was excellent on PDA, followed by water hyacinth’s media, Richard’s agar media and Martin’s media, good on Lenolin’s media, malt extract, Asthana & Hawker’s media and Sabouraud’s media. Sporulation was also fair on yeast glucose, Czapek’s media and maltose peptone but scarce on Miller’s and Mayer’s media. The media viz., water hyacinth (host leaf) extract failed to support excellent growth but induced significant sporulation. On various media, there was no relationship between vegetative growth and sporulation since Miller's and Mayer's media did not promote good sporulation even though they supported good growth. Singh et al. (2001) and Zhang et al. (2001) have reported similar results of outstanding growth and sporulation on PDA in *Alternaria alternata*.

Table 1. Effect of different nutritional media (solid media) on growth and sporulation of *Alternaria petroselini* (FCEC#38)

S. No.	MEDIA	GROWTH INITIATION AT 24 HRS	COLONY DIAMETER (MM) (MEAN±SD)			AVERAGE SPORULATION (PERML.)
			3 DAYS	5 DAYS	7 DAYS	
1.	Malt Extract	++++	36.4 ± 0.27	47.5 ± 1.45	56.9 ± 0.84	2.5×10^6
2.	Yeast Glucose	+++	32.7 ± 1.26	57.2 ± 0.53	68.8 ± 0.24	2.1×10^6
3.	Maltose Peptone	++	37.1 ± 0.21	43.7 ± 0.54	55.1 ± 0.44	1.3×10^6
4.	Czapek’s	++	34.2 ± 0.35	48.8 ± 1.47	58.3 ± 0.5	1.7×10^6
5.	Miller’s	+	34.7 ± 1.10	47.5 ± 0.68	63.5 ± 0.77	0.7×10^6
6.	Mayer’s	+	34.2 ± 0.82	47.7 ± 0.63	70.1 ± 0.46	0.3×10^6
7.	Martin’s	++++	37.5 ± 1.21	52.1 ± 0.32	68.1 ± 0.3	3.2×10^6
8.	Sabouraud’s	++++	34.5 ± 0.61	49.3 ± 0.19	59.7 ± 0.49	1.9×10^6
9.	Lenolin’s	+++	31.4 ± 0.69	41.3 ± 0.79	53.4 ± 1.39	2.5×10^6
10.	Richard’s	++++	29.1 ± 0.61	54.4 ± 1.39	63.8 ± 0.75	3.3×10^6

11.	PDA	+++++	37.4 ± 0.87	55.4 ± 1.43	72.1 ± 0.49	3.1×10^6
12.	Waterhyacinth's	+++	33.2 ± 0.64	47.5 ± 0.49	61.4 ± 0.42	3.3×10^6
13.	Asthana & Hawker	++	25.6 ± 1.09	43.6 ± 1.55	57.2 ± 0.98	2.2×10^6

Effect of nutritional media on biomass, toxin production and Phytotoxic effect by *Alternaria petroselini* (FCEC#38)

The fungus's maximum dry weight was noted on the PDA. Growth on Richard's medium and malt extract was noteworthy as well. Following this were the media of Martin, Sabouraud, Czapek, Lenolin, waterhyacinth extract, maltose peptone, and Mayer. Miller's media, Cohn's media, and yeast glucose all showed poor growth. There was no sporulation in the liquid media. Many diseases, including Alternaria and species that produce pycnidia, do not produce spores in liquid culture, according to Tebeest (1996). Singh et al. (2001) similarly found a similar lack of sporulation in liquid culture.

According to the findings in the Graph, *Alternaria petroselini* (FCEC#38) culture filterate cultivated on potato dextrose broth exhibited exceptional water hyacinth toxicity. When sprayed on water hyacinth, CFCF from potato dextrose broth killed all of the plants. Richard's broth, Sabouraud's broth, Mayer's, and Cohn's media came next. Other media's CFCF demonstrated minimal harm. According to Parveen and Kumar (2002).

Table 02. Effect of nutritional media on biomass and toxin production by *Alternaria petroselini* (FCEC#38).

S. No.	MEDIA	GROWTH INITIATION AT 24 HRS	CHANGE IN pH 21DAY	MYCELIAL DRY WEIGHT AFTER 21 DAYS *(in g/1)* (MEAN± SD)
1.	Malt Extract	++++	3.4	12.3 ± 0.5
2.	Yeast Glucose	+++	4.3	2.1 ± 0.8
3.	Maltose Peptone	++	7.3	4.2 ± 0.4
4.	Czapek's	++	4.3	5.6 ± 0.5
5.	Miller's	+	6.8	1.4 ± 0.4
6.	Mayer's	+	3.1	2.9 ± 0.8
7.	Cohn's	-	3.4	0.8 ± 0.2
8.	Martin's	++++	4.4	11.7 ± 0.3

9.	Sabouraud's	++++	5.3	12.4 ± 0.6
10.	Lenolin's	+++	4.7	6.5 ± 0.15
11.	Richard's	++++	4.8	12.9 ± 0.2
12.	PDA	+++++	5.7	13.4 ± 0.3
13	Waterhyacinth's	+++	4.4	8.08 ± 1.1

Graph 03. Phytotoxic effect of different nutritional media (liquid media) on water hyacinth

S. No.	MEDIA	PHYTOTOXIC EFFECT (in %)		
		1 day	3 days	7 days
1.	Malt Extract	7.4	20.2	57.3
2.	Yeast Glucose	12.1	16.4	51.4
3.	Maltose Peptone	9.7	32.4	53.2
4.	Czapek's	39.4	58.1	75.1
5.	Miller's	0.0	3.4	15.4
6.	Mayer's	42.2	47.5	84.5
7.	Cohn's	59.4	53.4	81.4
8.	Martin's	18.4	36.4	74.4
9.	Sabouraud's	29.4	62.4	80.6
10.	Lenolin's	12.4	13.4	33.5
11.	Richard's	63.3	72.4	99.8
12.	PDA	61.4	81.4	102.4
13	Waterhyacinth's	16.7	47.4	70.4

Conclusion

In this work, the culture medium was optimized based on Natural media, Semi synthetic media and Synthetic Media for growth of ***Alternaria petroselini*** **(FCEC#38)., which is a potential fungus for the production of bioherbicide.** On the basis of all observation, it is concluded that the effect of different nutrient medium was played a important role for the growth of ***Alternaria petroselini*** **(FCEC#38)** and the screening of Potato Dextrose media (solid and liquid) are essential for large scale production of biomass of the

***Alternaria petroselini* (FCEC#38)** for the management of **water hyacinth** weeds.

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