WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 8, 678-684. Research Article ISSN 2277– 7105

SCREENING OF CULTURE MEDIA ON THE GROWTH OF BIO-CONTROL AGENT *ALTERNARIA ALTERNATA* (FCWH#46)

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Article Received on
18 February 2018,
Revised on 11 March 2018,
Accepted on 2 April 2018,
DOI: 10.20959/wjpr20188-11788

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ABSTRACT

Effect of different media were tested against the growth of *Alternaria alternata* (FCWH#46) under *in-vitro* condition. The growths of fungi were tested in 13 different liquid and solid media. The growth characteristics such as color of colony and substrate, margin of colony were studied on the solid media. Maximum growth of Bio-control agent *Alternaria alternata* (FCWH#46) was observed at 7 days. After inoculation liquid and solid media continuous increasing growth in the Potato Dextrose Media.

KEYWORDS: *Alternaria alternata* (FCWH#46), Different media, Growth characters.

INTRODUCTION

Fungi are specific in their choice of food but same substances are the most important substance required by fungi with regard to their vegetative and reproductive growth. The nutrient requirements for good sporulation. Various media compositions also influence the different colony morphology of *Alternaria alternata* (FCWH#46). Morphological character is the classical approaches to distinguish fungal sp. that in one of the main requisite of fungal taxonomy.

Culture media is the major factor influencing fungal cultivation. A wide range of media is used for growing fungi. All fungi require several specific elements for growth and reproduction. The concentration of medium constituents determines the quality and quantity of growth and whether sporulation or vegetative growth will dominate. Media will affect colony morphology and color, whether particular structures are formed or not, and may affect whether the fungus will even grow in culture. A good culture medium supports high

sporulation and low mycelia growth. Generally, sporulation is favored by nutritional exhaustion. Natural media, in general are more favorable to growth and sporulation than synthetic ones.

Nutritional requirement of various fungi differ and there is no single medium or substrate, which can be universally suited for all the fungi. The genetic constitution of fungus determines what it can do but the expression of its potentiality is dependent upon the composition of the medium on which it grows and environment it is exposed to. Thus, various nutritional media were tested to select the best medium for growth, sporulation and toxin production by *Alternaria alternata* (FCWH#46).

Therefore the objective of this study effect on the growth of *Alternaria alternata* (FCWH#46) in different solid and liquid media.

MATERIAL AND METHODS

Some of the common synthetic, semi synthetic and natural media in solid and liquid form were used to growth the fungus. *Alternaria alternata* (FCWH#46) were isolated from the infected part of water hyacinth. After screening of the pathogen were maintained on potato dextrose agar media. Purified culture of the *Alternaria alternata* (FCWH#46) was inoculated in to the thirteen solid and thirteen liquid media –PDA, Richard's, Asthana & Hawker, Sabouraud's, Martin's, Miller's, Mayer's, Lenolin's, Czapek's, Maltose Peptone, Yeast Glucose, Malt Extract, Waterhyacinth.

The semi solid as well as liquid media were inoculated with 5mm agar disks of hyphal tips taken from 5 days old cultures. All these were done under perfect aseptic conditions inside an inoculation chamber. The radial growth of the colony in Petri plate was measured after 3, 5 and 7 days of incubation and in liquid case the average growth was observed by dry mycelia weight after 21 days and the color, colony and substrate margin of colony topography of mycelium were observed by naked eye.

RESULT AND DISCUSSION

(i) Effect of nutritional media on growth and sporulation of *Alternaria alternata* (FCWH#46)

The result presented in the Table 1 (i) and (ii), show that the radial growth of the pathogen at different time interval was found to vary significantly with respect to different media. There

was appreciable difference in morphology of *Alternaria alternata* (FCWH#46) grown on different media (Table 1(i)) Maximum radial growth was observed on PDA, which was at par with radial growth on Martin's media and yeast glucose. This was followed by Mayer's, Richard's media, Miller's media; water hyacinth's (hostleaf) extract media, Sabouraud's media, Czapek's, malt extract, Asthana and Hawker's media, maltose peptone and Lenolin's media. Differences in growth characteristics of *Alternaria alternata* (FCWH#46) on different media are shown in Table 1 (i).

Similar result of excellent growth has been reported on PDA in *Alternaria alternata* (FCWH#46) keissler by Singh *et al*. (2001), in *Plectosporium tabacinum* by, Zhang *et al.* (2001), Aneja and Singh (1989) observed that their isolate of *Alternaria alternata* (FCWH#46) from water hyacinth grew better on water hyacinth dextrose agar (WHDA) than PDA. In general natural media proved to be better for growth but they were not selected as basal media because of their inherent complexities: firstly they contain natural products and constituents of unknown composition and secondly these products and constituents vary qualitatively with the age and variety of the host tissue used. Richard's media was selected as basal medium for subsequent physiochemical studies because it could induce satisfactory vegetative growth and sporulation and it is comparatively inexpensive and simple in composition.

(ii) Effect of nutritional media on biomass and toxin production by *Alternaria alternata* (FCWH#46)

It is evident from the data recorded in the Table 1 (ii),(iii), that the nutritional media indeed plays an important role in the growth, sporulation and toxin production of the fungus. Maximum dry weight of the fungus was recorded on Potato dextrose agar (PDA). Growth was also significant on Richard's medium and malt extract. This was followed by Martin's media, Sabouraud's media, water hyacinth extract, Czapek's media, Lanolin's media, Maltose peptone and Mayer's media. Poor growth was seen in yeast glucose, Cohn's media and Miller's media. Sporulation was absent in the liquid medium.

According to Tebeest (1996) many pathogens, such as *Alternaria* or *pycnidia* producing species, do not produce spores in liquid culture. Similar absence of sporulation in liquid culture was also reported by Singh *et al*. (2001).

The data presented in Table 1 (iii), indicates that culture filtrate of *Alternaria alternata*

(FCWH#46) grown on potato dextrose broth showed excellent toxicity to water hyacinth. CFCF from potato dextrose broth when sprayed on water hyacinth caused 100% mortality of the plant. It was followed by Richard's broth, Sabouraud's broth, Mayer's and Cohn's media. CFCF of other media showed low toxicity. Praveen and Kumar (2004) reported maximum toxicity produced by *Alternaria triticina* in Richard's broth followed by glucose, glutamic acid, potato dextrose and Brown's broth. Richard's broth had been successfully used as a basal media for phytotoxin production by several *Alternaria* species (Nakatsuka *et al*., 1986); *Rhizoctonia solani* (Lakpale *et al*., 1996); *Colletotrichum gloeosporioides* (Sharma *et al;* 1989).

Table No. 1: Growth characteristics of *Alternaria alternata* (FCWH#46) in different Solid Media.

S. No.	MEDIA	GROWTH CHARACTERISTICS
1.	PDA	Colony effuse, grayish, floccose, mycelium not immersed, superficial
2.	Richard's	Colony cottony and wooly growth, pinkish with olivaceous green periphery.
3.	Asthana & Hawker	green margin, mycelium partly immersed, circular
4.	Sabouraud's	pinkish with circular green periphery, margin irregular
5.	Martin's	Loose circular, fair cottony growth, olivaceous black with green periphery Colony floccose, mycelium partly superficial,
6.	Miller's	Circular, loose, white, mycelium immersed.
7.	Mayer's	Colony effuse, cottony, mycelium superficial, pink to yellowish, circular
8.	Lenolin's	Colony not effuses lightly bluffy, light grey in colour, irregular shape, and mycelium superficial.
9.	Czapek's	Colony fluffy, white cottony growth, mycelium superficial
10.	Maltose Peptone	Colonies forming concentric rings of black colour to white colour. Mycelium partly immersed
11.	Yeast Glucose	Colony effuse, light grey in colour, mconspicuous
12.	Malt Extract	Colony fluffy, cottony, mycelium superficial, pink to yellowish
13.	Waterhyacinth	Colony hairy, white to backish, mycelium immersed circular. Light cottony growth, not compact, white with

Table No. 1: Effect of different nutritional media (solid media) on growth and sporulation of *Alternaria alternata* (FCWH#46).

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	++++	37.1 ± 0.25	48.4 ± 1.25	57.8 ± 0.54	2.5×10^6
2.	Yeast Glucose	+++	33.4 ± 1.26	57.3 ± 0.53	67.8 ± 0.24	2.1×10^6
3.	Maltose Peptone	++	32.2 ± 0.28	43.7 ± 0.46	54.1 ± 1.45	1.3×10^6
4.	Czapek's	++	33.2 ± 0.37	45.8 ± 1.21	57.3 ± 0.49	1.7×10^6

5.	Miller's	+	34.7 ± 1.10	48.5 ± 0.97	61.3 ± 0.85	0.7×10^6
6.	Mayer's	+	32.4 ± 0.74	45.7 ± 0.75	68.4 ± 0.46	0.3×10^6
7.	Martin's	++++	38.5 ± 1.17	51.6 ± 0.42	67.5 ± 0.27	3.2×10^6
8.	Sabouraud's	++++	35.4 ± 0.59	48.2 ± 0.15	58.4 ± 0.46	1.9×10^6
9.	Lenolin's	+++	32.4 ± 0.73	42.1 ± 0.86	52.4 ± 1.43	2.5×10^6
10.	Richard's	++++	28.9 ± 0.63	54.2 ± 1.42	64.1 ± 0.74	3.3×10^6
11.	PDA	+++++	36.2 ± 0.88	57.4 ± 1.63	71.2 ± 0.53	3.1×10^6
12.	Waterhyacinth's	+++	34.1 ± 0.62	45.7 ± 0.51	62.5 ± 0.83	3.3×10^6
13.	Asthana & Hawker	++	26.5 ± 1.08	42.7 ± 0.87	56.5 ± 1.07	2.2×10^6

Table No. 1: Effect of different nutritional media (liquid media) on biomass production of *Alternaria alternata* (FCWH#46).

S. No.	Media	Growth Initiation at 24 Hrs	Change in Ph 21 day	Mycelial Dry weight After 21 days (in g/1) (mean± sd)
1.	Malt Extract	++++	4.3	13.2 ± 0.4
2.	Yeast Glucose	+++	5.2	1.8 ± 0.7
3.	Maltose Peptone	++	8.2	5.1 ± 0.3
4.	Czapek's	++	5.2	6.5 ± 0.4
5.	Miller's	+	7.7	1.1 ± 0.3
6.	Mayer's	+	2.7	3.1 ± 0.8
7.	Cohn's	-	2.5	1.2 ± 0.1
8.	Martin's	++++	5.3	12.6 ± 0.4
9.	Sabouraud	++++	6.1	11.3 ± 0.7
10.	Lenolin's	+++	5.5	5.1 ± 0.17
11.	Richard's	++++	5.6	13.8 ± 0.3
12.	PDA	+++++	6.3	14.2 ± 0.4
13	Waterhyacinth	+++	5.3	9.07 ± 1.0

Fig. 1: Growth of *Alternaria alternata* on different liquid media.

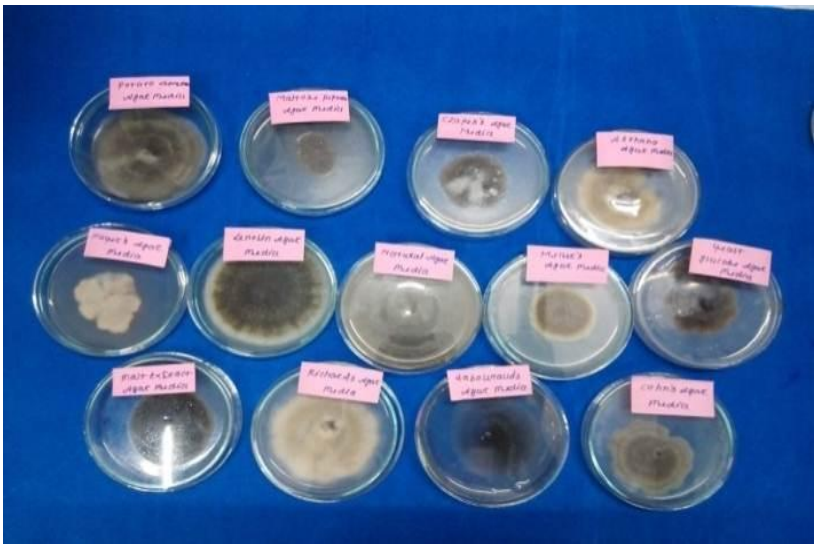

Fig. 2: Growth of *Alternaria alternata* on different Solid media.

CONCLUSION

From the above result, it can be concluded that the potato dextrose agar medium Influences the best radial growth were observed in solid and liquid media. Potato dextrose broth medium shows maximum dry mycelium weight. This study will be helpful for growth parameters of the test fungus.

ACKNOWLEDGEMENT

I thankful to our Supervisor Dept. of P.G Studies in Biological Science, Rani Durgavati University, Jabalpur. (M.P). I also expresses our deepest gratitude towards Madhya Pradesh Council of Science & Technology (MPCST), Bhopal for providing us with the most important financial support.

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