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Comparative Impact of Physico- Chemical and Nutritional Parameters on some Phytopathogenic Fungi Isolated from the Phyllosphere of Diseased Tea Leaf (Camellia sinensis L. O. Kuntze).

KEYWORDS Pathogenic fungi, Carbon, Nitrogen, temperature, pH, salt tolerance, Camellia sinensis.

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ABSTRACT *The present study has been evaluated to observe the comparative impact and susceptibility in different physicochemical and nutritional parameters on the mycelial growth of some phytopathogenic fungi viz.; Pestalotiopsis sp, Colletotrichum gloeosporioides, F. solani, Alternaria alternata and Cladosporium cladosporoids isolated from the phyllosphere of diseased tea leaf (Camellia sinensis L. O. Kuntze). The result implied different cultural activities of spore germination and mycelium growth on different culture media, carbon and nitrogen sources. The pathogens were categorized according to temperature, pH level and salt tolerance capacity. The result indicated fast growth of all the pathogens on Potato Malt Agar medium followed by Potato Dextrose Agar but change in colony morphology. Among the carbon sources tested dextrose combined medium has produced highest mycelium by Pestalotiopsis sp and Colletotrichum gloeosporioides and in case of nitrogen sources tested Ammonium nitrate has yielded highest mycelia followed by malt extract, $NaNO_3$, KNO_3, Ammonium sulphate and peptone. The experimental results of physical parameter implied that the pathogen showed batter growth in 25˚ C to 35˚C temperature which can be categorized as mesophiles and among the observed pH level tolerance test maximum growth was observed at pH 5.5. Alternaria alternata and F. solani showed highest tolerance (2.5%) of NaCl salt.*

Introduction:

Leaf is a habitat for the microorganisms which is exposed to the environment and tolerates all the extremes of the nature like heavy rainfall, high temperature and moisture. Warm weather and frequent rainfall is a suitable habitat for the growth of phytopathogenic fungi. The frequency of the disease occurrence is mainly due to considerable production, fragmentation as well as inappropriate hygiene conditions. The development of pathogens, their virulence and spreading velocity, to a considerable extend depend on conditions prevailing during the cultivation practices. These conditions are, among others, temperature and cover pH (Maszkiewicz et al, 2006; and Fletcher and Gaze 2008).

Alternatively, an effective screening process can be achieved through systematic manipulation of culture conditions for small number of promising organisms. In fact culture condition has a major impact on the growth of microbes and the production of microbial products. As far as culture conditions concerned, there is usually a dilemma in achieving maximal growth rate and maximal antibiotic yields because condition that allows fast growth could be unfavorable to metabolite production (Audhya and Russell 1974, Frisvad and Samson 1991).

The yield of bioactive compounds can sometimes be substantially increased by the optimization of Physical (temperature, salinity, pH and light) and chemical factors (media components, precursors and inhibitors) for the growth of microbes (Calvo et al 2002; Llorens etal 2004).

Environmental factors such as relative humidity and temperature were reported to have a profound influence on the ineffectiveness of a variety of fungi. Growth rate of fungi vary depending on temperature and relative humidity. The optimum growth temperatures for the majority of fungi were found to fall between 25˚C to 30˚ C. Above 40˚ C, the growth was poor and in some cases mortality was observed (Sharma and Razak, 2003)

Looking at the adverse effect of the pathogens on the yield and annual crop loss, it is important to study the occurrence, behavior and physiological characteristics of the pathogens in vitro for their control in field condition.

Material and Methods:

Isolation of pathogenic fungi:

The pathogenic fungi were isolated from diseased tea leaves of different agro climatic conditions. Isolation of the fungai was carried by adopting standard serial dilution method and spread plate technique (Waksman, 1961). The leaves were surface sterilized with 0.1% Mercuric chloride ($HgCl_2$) and then five discs each of 5mm diameters were transferred to 100ml sterile water in Erlenmeyer conical flask (250ml) and shaken vigorously. From the suspension, the serial dilution up to 10^2, 10^3 and 10^4 were prepared. 1ml aliquot was inoculated in sterilized molten cooled PDA in sterilized petridish.

The leaf impression method was also employed. It involved the direct pressing of the leaf discs after surface sterilization from both dorsal and ventral surfaces on the solidified medium to obtain the impression. The plates were incubated at 25°C for 5days (Aneja, 2003). Identification was based on morphological and cultural observations, (Barnett Hunter, 1972, Domsch et al, 1980 and Ellis, 1971, 1976).

Nutritional parameters

Influence of culture media on fungal growth:

The mycelial growth of the fungi was evaluated on eight different culture media viz. PDA, Czepex Dox Agar (CDA), Fungal Agar (FA), Potato Malt Agar (PMA), Corn Meal Agar (CMA), Malt Extract Agar (MEA), Oat Meal Agar (OMA),

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Water Agar, Tea leaf extract Agar(TLEA) and Tea stem extract Agar(TSEA). The plates containing all the culture media were inoculated with mycelial disk of all isolates aseptically in three replicates for each isolate with proper labeling and incubated at 28˚C±2. The colony diameter of each was measured along two axes perpendicular to one another at 0, 2, 4 and 6 days after inoculation. The radial growth rate was averaged to give a final value.

Influence of carbon source on the growth of fungi:
Effect of carbon sources were studied by taking Czepex Dox Mediaum as basal medium and different carbon sources tested was supplemented in it in equivalent proportion of the carbon compound present in the basal medium. The control medium did not contain any carbon compound. Then the flasks (250ml) containing 100ml broth in each was inoculated with mycelial disk of 6mm diameter and incubated at 28±2˚C for 10 days and the dry weight of the mycelium was taken and recorded.

Influence of nitrogen source on the growth of fungi:
The effect of nitrogen sources was studied by taking Czepex Dox Mediaum as basal medium and the nitrogen compound of the basal medium was replaced with different nitrogen sources. The control medium was kept without any nitrogen sources. The conical flasks(250ml) containing 100ml of broth in each was inoculated aseptically with 6mm diameter of mycelial disk cut out from an actively growing colony of the fungal isolates and incubated at 28±2˚C for 10 days and the dry weight of the mycelium was taken and recorded.

Physico- chemical parameters
Effect of temperature:
Effect of temperature on mycelial growth and sporulation of the isolates was observed by growing in Potato Dextrose Broth at different temperature regimes of 10˚C, 15˚C, 25˚C, 30˚C, 40˚C and 45˚C. The conical flask containing 30ml of PDB was inoculated with mycelial disc of 6mm diameter of each isolates and incubated at different temperature regimes for 10 days and the dry weight of the mycelium was taken and recorded (Aneja, 2003).

Effect of pH on mycelial growth:
Effect of pH on mycelial growth was studied by growing the fungal isolates in Potato Dextrose Broth mixed with an equal volume of the buffer to give desired pH in medium. The pH was obtained over the ranges 3-7 and 7-9 with citrate phosphate (0.1 M solution of citric acid, 0.2 M solution of $Na_2HPO_4.7H_2O$) and Tris (hydroxymethyl) aminomethane (0.2 M solution of Tris aminomethane, 0.2 M HCL) buffers respectively by following the method of (Gomori, 1955).The PDB medium and the buffers were autoclaved separately and aseptically mixed during cooling. Then the flask containing 30ml of broth was inoculated with mycelial disc of 6mm diameter of each isolates and incubated at 28˚C in dark for 10 days, thereafter, dry weight of the mycelium was recorded.

Effect of salinity on mycelial growth:
To test the salt tolerance, different concentration of NaCl was prepared viz.; control, 0.5%, 2.5%, 5%, 7.5% and 10%. The different salt concentrations were poured in to 250ml conical flasks containing 100ml of PDB. The flasks were sterilized in autoclave and after cooling inoculated with the test fungal isolates, labeled properly and incubated at 28˚C for 10days and the dry weight of the mycelium was taken and recorded.

Results and Discussion:
Influence of culture media on fungal growth:
It was apparent that the phytopathogens exhibited batter growth in rich nutrient medium. In Potato Malt Agar all the fungal isolate produced maximum growth where as lowest growth was observed on Water Agar. PMA was most suitable growth medium followed by PDA, FA and Tea Leaf Extract Agar. Fusarium solani has highest colony diameter (7.7± 0.2cm) on Potato Malt Agar, (6.7± 0.21cm) in Potato Dextrose Agar and lowest colony diameter of (1.4± 0.22cm) on Water Agar medium. Cladosporium cladosporoides has highest colony diameter of (3.26± 0.27cm) on PMA medium. (Table-1).

Influence of carbon sources on mycelial growth:
Dextrose (Hexose) was the best carbon source for mycelial growth. C. gloeosporioides produced highest mycelium growth (6.9± 0.13) in Dextrose followed by Maltose (Table-2). Bhatnagar and Prasad (1968) suggested that among the carbon sources monosaccharide were better but among disaccharides, maltose was proved to be best for growth and sporulation of Fusarium solani f. aurantifoliae responsible for Lime twig disease. Chaturvedi (1965) has reported that, glucose, fructose, maltose and starch supported good sporulation of C. gloeosporioides isolated from the incitant of leaf spot of Polycias balijuriana. Hegde (1990) C. gloeosporioides isolated from arecanut shows good growth in glucose and dextrose among the tested carbon sources. Naik et al (1988) reported sucrose as the best carbon source followed by glucose and dextrose for the growth of C. gloeosporioides isolated from betel vine causing anthracnose disease.

Influence of nitrogen sources on mycelial growth:
The mycelial expansion and sporulation of F. solani was maximum in ammonium nitrate (1.13±0.02), followed by C. gloeosporioides (1.2± 0.10) in malt extract (organic) (Table-3). Wasantha Kumara and Rawal (2008) observed aspartic acid as batter nitrogen source for the growth and potassium nitrate, ammonium nitrate or sodium nitrate for the sporulation of C. gloeosporioides isolated from papaya causing anthracnose disease. Manjunatha Raw and Rawal (2002) suggested that Ammonium nitrate as better nitrogen source for the mycelial growth and sodium nitrate favored batter sporulation of C. gloeosporioides isolated from grapevine. Khandare(2012)reported all the nitrogen sources used were stimulant but ammonium nitrate was most stimulant for the pathogen Alternaria alternata.

Growth of fungi at different temperatures:
The phytopathogens exhibited maximum mycelial growth from 25˚C to 30˚C and can be categorized as mesophiles. All the isolate showed minimum growth from 15˚C (weak psychrophiles). Alternaria alternata shows slender growth at high temperature above 35˚C and 40˚C (weak thermophiles), (Table-4). Sangeetha (2002) reported that different isolates of C. gloeosporioides isolated from mango shows maximum growth at temperature range of 25 to 30˚C and good sporulation was observed at range 25 to 28˚C.

Growth at different pH level:
The mycelial growth of the phytopathogens in different pH levels has demonstrated that there was no growth of mycelium and sporulation below pH 2.0 and above pH 9.5. Mycelial growth started from pH 2.5 and maximum growth was observed in the range of pH 5.5 to 6.0(Fig-1).

Salinity tolerance test:
The pathogenic isolates examined under different con-

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centration of NaCl, F. solani and Alternaria alternata have shown highest tolerance up to 2.5% concentration, above this no growth in fungal colony was observed.

Table-1 Growth of pathogenic fungi in different culture media.

Culture media	Isolates(growth in cm)				
	Pestalotiopsis sp	Colletotrichum gloeosporioides	Alternaria alternata	Cladosporium cladosporoids	Fusarium solani
PDA media	5.60± 0.29	5.1± 0.26	3.0± 0.25	2.7± 0.15	6.7± 0.21
Czepex Dox Agar	3.53± 0.25	3.08± 0.13	3.6± 0.74	2.5± 0.34	4.87± 0.76
Fungal Agar	4.3± 0.45	4.22± 0.11	3.4±0.13	2.56± 0.4	5.45± 0.13
Potato Malt Agar	6.5± 0.09	6.6± 0.29	3.58± 0.27	3.26± 0.27	7.7± 0.2
Water Agar	0.83± 0.12	1.08± 0.11	1.15± 0.13	0.62± 0.11	1.4± 0.22
Tea Leaf Extract Agar	4.4± 0.17	4.3± 0.08	2.3± 0.27	2.2± 0.3	4.7± 0.15
Tea Stem Agar	3.85± 0.11	3.6± 0.12	2.3± 0.01	2.3± 0.3	4.7± 0.27

± S.D (Average of three replicates)

Table-2 Influence of carbon sources on mycelial growth of fungi:

Carbon sources	Pestalotiopsis sp	Colletotrichum gloeosporioides	Alternaria alternata	Cladosporium cladosporoids	Fusarium solani
Dextrose	4.67± 0.41	6.9± 0.13	3.5± 0.21	3.2± 0.47	5.5± 0.26
Galactose	3.50± 0.30	5.73± 0.10	2.8± 0.20	2.4± 0.50	5.4± 0.20
Mannitol	2.6± 0.50	3.70± 0.13	2.60± 0.23	2.30± 0.20	3.50± 0.34
Sucrose	2.60± 0.25	3.40± 0.40	2.50± 0.20	2.52± 0.35	3.70± 0.23
Maltose	3.40± 0.33	6.50±0.07	2.50± 0.30	2.40± 0.35	3.70± 0.15
Control	2.12± 0.16	2.34± 0.30	1.6± 0.12	1.63± 0.23	2.10± 0.40

± S.D (Average of three replicates)

Table-3 Influence of nitrogen sources on mycelial growth of fungi:

Nitrogen sources	Pestalotiopsis sp		Colletotrichum gloeosporioides		Alternaria alternata		Cladosporium cladosporoids		Fusarium solani	
	Dry wt.	Final pH	Dry wt.	Final pH	Dry wt.	Final pH	Dry wt.	Final pH	Dry wt.	Final pH
Ammonium sulphate	0.97± 0.01	5.3	1.01± 0.01	5.3	0.91± 0.04	5.5	0.76± 0.03	5.3	1.05± 0.03	5.0
Ammonium nitrate	1.10± 0.05	5.8	1.04± 0.03	5.7	0.98± 0.03	5.8	0.86± 0.02	5.6	1.13± 0.02	5.5
Sodium nitrate	1.02± 0.02	5.7	1.01± 0.01	5.5	0.95± 0.02	5.3	0.82± 0.01	5.4	1.12± 0.13	5.3
Potassium nitrate	0.97± 0.01	5.1	0.98± 0.01	5.4	0.90± 0.07	5.7	0.77± 0.01	5.3	1.10± 0.08	5.1
Peptone	0.9± 0.04	5.3	1.0± 0.14	5.0	0.9± 0.08	5.7	0.78± 0.06	5.5	1.07± 0.20	5.2
Malt Extract	1.0±0.07	5.6	1.2± 0.10	5.2	0.9± 0.06	5.3	0.83± 0.05	5.2	1.10± 0.06	5.4
Control	0.1± 0.03	5.5	0.1± 0.03	5.2	0.1± 0.03	5.3	0.12± 0.04	5.5	0.12± 0.04	5.3

± S.E (Average of three replicates)

Table-4. Effect of temperature on fungal growth:

Name of isolate	Incubation temperature					
	Psychrophiles		Mesophiles		Thermophiles	
	5°C	15°C	25°C	30°C	35°C	40°C
Fusarium solani	-	+	+++	+++	+	-
Cladosporium cladosporoids	-	+	++	+++	-	-
Altrenaria alternata	-	+	++	+++	++	+
Pestalotiopsis sp	-	+	+++	+++	+	-
Colletotrichum gleosporioides	-	+	+++	+++	+	-

No growth (-), Slow growth (+), Moderate growth (++) and Very good growth (+++).

Fig-1. Growth in different pH level:

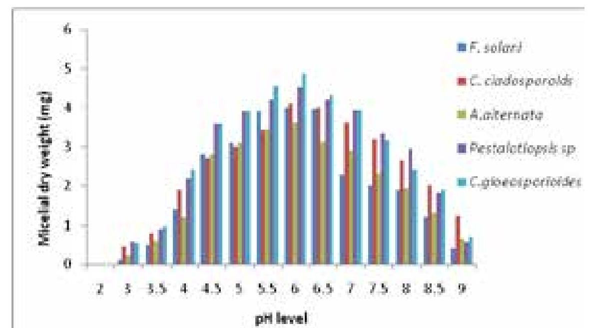
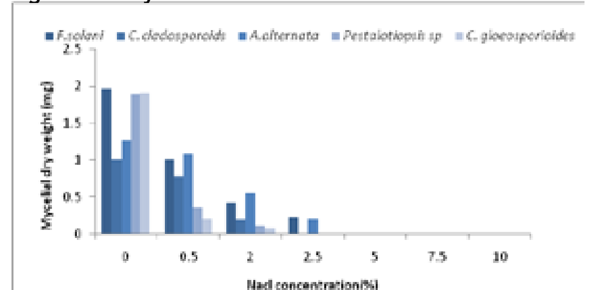


Fig-2. Salinity tolerance test:



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