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Effect of Culture Media and Environmental Conditions on Mycelium Growth and Sporulation of *Chrysosporium queenslandicum*

Tarun Kumar Kumawat[1], Anima Sharma[1]*, Seema Bhadauria[2]

[1]Department of Biotechnology, JECRC University, Jaipur, Rajasthan, India
[2]Department of Microbiology, JECRC University, Jaipur, Rajasthan, India

Abstract : The influence of various culture media and environmental factors play an imperative role in the growth of mycelia and sporulation of keratinophilic fungi. Fungi grow up best at optimal temperature, pH, humidity and culture media. The extremely high and low temperature, as well as pHs, reduce the growth. The study was aimed at determining the effect of optimal parameters (incubation temperature, pH and culture media) for the mycelium growth and sporulation of *Chrysosporium queenslandicum* (KU560575) isolated from the poultry farm of Rajasthan. The fungus was identified by morphological and molecular characteristics of the ITS1-5.8S-ITS2 rDNA region. The growth and sporulation was evaluated on different temperature regimes i.e. 5, 15, 25, 35, 45, 55 ^{o}C, pH 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and culture media *i.e.* Sabouraud's dextrose broth (SDB), Richard's synthetic broth (RSB), Czapek dox broth (CDB), Mannitol salt broth (MSB), Yeast extract broth (YEB), Malt extract broth (MEB). *C. queenslandicum* showed their maximum mycelium growth at 25^{o}C (1.321±0.08 gm) with the best sporulation at 25-35^{o}C. On the other hand, in the relation to pH maximum growth and best sporulation was recorded at pH 7.0 (1.459±0.05 gm). The fungus grew maximum on MEB (1.007±0.02 gm) followed by SDB (0.807±0.08 gm), but least grew on the YEB.
Key words: Environmental factors, keratinophilic fungi, *Chrysosporium queenslandicum.*

Introduction:

Fungi are the second biggest group of microorganisms after the inscects[1]and capable to colonize a extensive variety of different substrata. Fungi are spread all over the world because they easily dispersed at different environmental conditions[2]. It can adapt and grow under best optimal conditions of pH, temperature, nutrient availability and humidity[3].Keratinophilic fungi play vital role in the biodegradation of keratinous substrates with the help of keratinase enzyme[4]. A very large number of keratin-containing materials such as wool, feathers, animal hair, horns, etc are generated from the poultry processing plants, leather industries, and slaughterhouses in huge amounts and remains in the environment as a recalcitrant solid waste[5-8]. Keratinases are the industrially important proteolytic enzymes capable of hydrolyzing highly rigid keratin which is recalcitrant to commonly known proteases such as trypsin, pepsin and papain[9]. Soil is the natural reservoir for microorganisms[10]and keratinophilic fungi were found in humid soils which are rich in organic or keratin matter. Salty soils and roadside soils proved to be poor sources of keratinophilic fungi[11, 12].

Chrysosporium queenslandicum belongs to the family Onygenaceae and isolated first time from the feathers of domestic fowl Cunnumala, Southern Queensland, Australia[13]. The fungus vigorously colonizes and decomposes the human hair in the culture[14]. This is thermotolerent and also recorded from various habitats such as public park soil and sheep hairs[15, 16]. *C. queenslandicum* producing fungal nail infection (onychomycosis)

and cause a disseminated mycotic alteration in skin, lungs and liver in male garter snake (*Thamnophis*)[17]. Antifungal compound "Queenslandon" and antibacterial compounds "chrysoqueen (1) and chrysolandol (2)" related to members of the dihydronaphthaquinone group was produced by *Chrysosporium queenslandicum* IFM 51121[18, 19].

Various environmental factors such as pH, temperature play an important role in the growth and sporulation of keratinophilic fungi[20]. These conditions affecting the sexual reproduction in keratinophilic fungi[21].Temperature is the major abiotic parameter which determine the growth of micro-organism[22]. Sporulation and metabolic activities of fungi in culture media are greatly influenced by varying temperature. Most of the fungi require to grow at temperatures ranging from 15°C to 35°C[20]. The most favorable temperature for mycelium production was same on solid and liquid culture media. pH of the culture media considerably effect the growth of fungi by its action on the cell surfaces and on the availability of nutrients[23]. Studies on pH reveal that fungi grow at neutral to the weak acidic environment, with the maximum production of dry mycelial weight[24, 25].

However, little information is available on the role and influence of environmental factors on the mycelial growth and sporulation of keratinophilic fungi. In the present study author investigate the effect of temperature, pH and culture media, on mycelium growth and sporulation of *C. queenslandicum.* This fungal strainis a potential keratin degrader and also used in the production of antifungal and antibacterial compounds.

Material and Methods

Isolation and Identification of *Chrysosporium queenslandicum:*

The isolation of keratin degrading fungi was carried out by using hair bait technique[26]. After 21 days inoculum from fungus growth was taken and placed & maintained on plate of Sabouraud's dextrose agar (SDA).Identification was done by morphological as well as molecular characteristics.

DNA Sequence Analysis:

Molecular characteristic of the strain was achieved by DNA sequence analysis of the ITS1-5.8S-ITS2 region. First, the fungus was grown in flask containing Sabouraud's dextrose broth and incubated at 28^{o}C for several days using an orbital shaking incubator (Remi CIS-24 Plus). After the colony growth, the genomic DNA was extracted by the Miniprep protocol with mild modification[27]. The frozen mycelium mass was smashed by mechanical pressure using sterile mortar piston and liquid nitrogen. The powder was mixed with lyses buffer and the DNA was extracted. The ITS1-5.8S-ITS2 rDNA was amplified using ITS4 and ITS5 universal primers[28]. Amplification was performed in 50 μL master mixture reaction volumes containing 5 μL of 10x buffer, 1 μL of dNTP (10 mM), 0.5 μL of ITS4 primer (10 pm), 0.5 μL of ITS5 primer (10 pm), 0.5 μL of Taq Polymerase (3.0 U) with the addition of $MgCl_2$ (15 mM) and 42.5 μL Milli Q Water with DNA Sample. The PCR reaction was carried out using eppendorfs Mastercycler Gradient Thermal Cycler with the following conditions: initial de-naturation at 94^{o}C for 10 minutes, 35 cycles of (1 minute at 94^{o}C for DNA de-naturation, 30 seconds at 56^{o}C for annealing and 1 minute at 72^{o}C for extension) final extension at 72^{o}C for 10 minutes and storage at 4^{o}C. Negative controls were also used in each set of reactions. The final products were analyzed by electrophoresis on 1.5% agarose (Himedia) and stained with 0.5 μg mL^{-1} ethidium bromide. The PCR products of the expected size were sequenced using ITS4 and ITS5 primers in an Applied Biosystem ABI Prism 3700 DNA analyzer at PGIMER, Chandigarh, India. Similarity analysis of the nucleotides was performed by using the web-based blasting program, basic local alignment search tool (BLAST) searches against sequences available in GenBank[29]. After performing the morphological traits and DNA sequence analysis the organism was confirmed as *Chrysosporium queenslandicum.*

Effect of Temperatures and Various pH on Mycelial Growth and Sporulation:

The Sabouraud's dextrose broth (SDB) medium was used for the study of the influence of different temperatures and pH. For temperature, the 100 ml. of culture media were prepared in 250 ml conical flasks. 10 mm discs of mycelia culture of *C. queenslandicum* was inoculated in the experimental flasks and incubated at different temperatures (5^{o}C to 55^{o}C at intervals of 10^{o}C) in the incubator. For pH, the 100 ml. of culture media were prepared in 250 ml conical flasks and was adjusted to different pH, ranging from 4.0 to 10.0, by adding 1N HCl or 1N NaOH. 10 mm discs of mycelia culture of *C. queenslandicum* was inoculated in the experimental

flasks and incubated at 28±2°C. Each treatment was replicated thrice. Mycelial dry weight and sporulation was recorded after 14 days.

Effect of Culture Media on Mycelial Growth and Sporulation:

Myceliumgrowth and sporulation of *C. queenslandicum* was studied on six culture media, *i.e.* Sabouraud's dextrose broth (SDB), Richard's synthetic broth (RSB), Czapek dox broth (CDB), Mannitol salt broth (MSB), Yeast extract broth (YEB), Malt extract broth (MEB). The 100ml. of culture media were prepared in 250 ml conical flasks and final pH was adjusted to 6.5. They were later inoculated aseptically with a 10 mm actively grown culture disc of the *Chrysosporium queenslandicum*. The flasks were incubated at 28±2°C in the incubator in triplicates. The mycelium dry weight and sporulation were recorded after 14 days.

Assessment of Dry Weight of the Fungus and Sporulation:

For assessing the growth of the fungus, mycelial mat was collected after the incubation days by filtering them through preweighed Whatman no. 1 filter paper individually. It was dry inside an incubator at a temperature of 50±2°C until a constant weight was obtained.

The actual weight of dry fungal mycelium was then calculated using the formula[30]:

Weight of mycelium = (Weight of filter paper + Weight of Mycelium) – (Weight of filter paper)

The degree of sporulation of fungi was determined using standard methods as recommended by Wilson and Knight[31]; Tuite[32].

pH variation:

The final pH of the culture filtrate was taken after 14 days using a digital pH meter (Model 181, Electronics India). A standard curve was prepared for the pH of culture filtrate over different media and temperature.

Results

In this study, the isolated strain yielded a unique PCR amplification. The sequence of ITS1 - 5.8S - ITS2 rDNA region were 517 bp. The ITS data of the isolated strain were identical to the ITS data of *C. queenslandicum* and pair wise alignment data showed more than 99% identities, which confirmed the identification of the *Chrysosporium queenslandicum* strain. GenBank accession number for isolated fungus was KU560575.

Effect of different temperature, pH and liquid culture media on *C. queenslandicum* growth was analyzed by dry mycelium weight and sporulation In case of the effect of temperature, the optimal temperature for mycelial growth was 25°C (1.321±0.08 gm). At lower and higher temperature, the mycelial growth and spore production was poor. Maximum sporulation was recorded at 25 and 35°C. The initial pH was floated towards the neutrality or an alkaline range after incubation except 15°C (Table: 1).

Table: 1. Average dry weight of mycelium and sporulation of *Chrysosporium queenslandicum* at different temperature regimes (Initial pH 6.5)

Temperature	Mycelium dry weight (gm)	pH of the filtrate	Sporulation
5°C	0.123±0.01	6.80	+
15°C	0.780±0.09	6.12	++
25°C	1.321±0.08	7.36	++++
35°C	1.103±0.10	6.89	++++
45°C	0.710±0.06	6.62	+
55°C	0.470±0.03	7.36	+

Note: Mean±SD (n=3); Sporulation grades: + = Poor growth and sporulation, ++ = fair growth and sporulation, +++ = Good growth and Sporulation

In case of influence of Hydrogen ion concentration, pH 6-8 was suitable for the mycelium growth. Maximum sporulation was recorded at neutral pH. It was also noticed that in high alkaline media the initial pH drifted towards the neutrality (Table: 2).

Table: 2. Average dry weight of mycelium and sporulation of *Chrysosporiumqueenslandicum* at different pH regimes (Initial temperature 28±2°C)

pH	Mycelium dry weight (gm)	pH of the filtrate	Sporulation
4.0	0.329±0.07	4.16	+
5.0	0.721±0.05	4.91	+
6.0	1.002±0.11	5.01	++
7.0	1.459±0.05	6.43	++++
8.0	1.290±0.10	7.38	+++
9.0	0.981±0.01	8.05	++
10.0	0.773±0.06	7.64	++

Note: Mean±SD (n=3); Sporulation grades: + = Poor growth and sporulation, ++ = fair growth and sporulation, +++ = Good growth and Sporulation, ++++ = Excellent growth and Sporulation

In the present research, the rate of growth of *C. queenslandicum* was compared in various liquid culture media types viz. SDB, RSB, CDB, MSB, YEB and MEB. The results showed that MEB (1.007±0.02 gm) is the most suitable liquid culture media followed by SDB (0.807±0.08 gm) for the growth of *C. queenslandicum.* Limited mycelial growth was observed in YEB & MSB, and moderate mycelial growth was observed in the CDB and RSB (Figure: 1). Media significantly affected mycelial growth of *C. queenslandicum.*

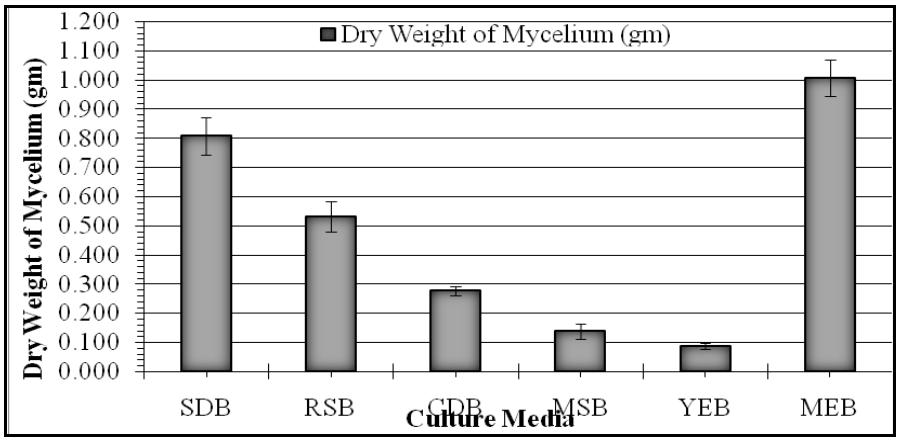

Figure: 1. Influence of culture media on the mycelium growth of *C. queenslandicum*

Discussion

Every fungus has a definite range and it can adapt and grow under various optimum conditions. Temperature is the most important physical condition for regulating the growth and reproduction of keratinophilic fungi[33]. The high temperature could lead to fungal cell rupture, loss of membrane or damage to the intra-cytoplasmic compounds[34].

In case of the effect of temperature, the optimal temperature for mycelial growth was 25°C and maximum sporulation was recorded at 25 and 35°C. Van Oorschot[35] reported the growth temperatures for *Chrysosporium queenslandicum* and presented the minimum temperature (10°C), optimum growth temperature (25°C) and maximum temperature (35°C) for the growth. Vissiennon[17]reported the first description of a *Chrysosporium queenslandicum* infection in a garter snake thatand observed that this fungus grew well at 28°C, the optimal temperature of the animal. Sharma and Sharma[20] investigate the effect of temperature and humidity on *Chrysosporium tropicum* and found that *C. tropicum* showed maximum growth at 30°C temperature, but the excellent sporulation was observed at 25°C to 35°C temperature. Reetha[232] studied that *Trichoderma harzianum* was grown faster at 25-30°C and it grow very slowly at above 35°C and there is no

growth at 45ºC. Optimum temperature of *Trichoderma* was found between 25 to 30ºC approx 28ºC by radial growth. Ibrahim[36] studied the effects of temperature on the growth of *Helminthosporium fulvum* and observed maximum growth at 30˚C temperature after 4 days of incubation. The growth of the fungus was drastically reduced below 15˚C and started to decline above 35˚C, as these temperatures did not favor the growth of the fungus.

In case of effect of Hydrogen ion concentration, mycelial growth and sporulation was highest at pH 7.00. It was evident from the present results that the *Chrysosporium queenslandicum* changed the pH of the medium by the end of the incubation days. Reetha[22] studied that the maximum growth of *Trichoderma harzianum* was observed at pH 7-7.5 and the minimum growth was observed at pH 5. Kotwal[37] recorded that, *Metarhizium anisopliae* was found best and maximum mycelial growth with abundant sporulation at pH 5.5. Saha[24] reported that the optimum pH for growth of *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl was at the range of pH 5.5-6.5. Abubakar[23] reported the highest mean of dried mycelial weight (355.67mg) was in pH 4.0 broth medium followed by 353.3mg in pH 7.0 of *Aspergillus parasiticus*. The lowest mycelia dry weight (302.73mg) was obtained in pH 10.0 broth medium. pH 5.0 produced the highest spores per ml (8.33 × 107), followed by pH 7.0 (7.67×107). The lowest spore formation of 2.83×107 was recorded at pH 10.0.

In the present investigation, different types of culture media affected the mycelial growth rate and conidial production in *Chrysosporium queenslandicum*. The results showed that MEB is the most suitable liquid culture media followed by SDB. Furthermore, in this study, when the fungus was cultured in MSB and YEB there was limited mycelial growth and sporulation (Figure: 1). Fungi are susceptible to nutritional factor and little variation in this factor may induce differences in their morphological characters, growth, and sporulation[39]. Gupta[38] studied the growth of keratinophilic fungi on five different media after seven days of incubation on 28^{o}C. Out of all media, SDA, found the best for all *Chrysosporium carmichaelii, C. georgii, C. indicum, C. keratinophilum, C. merdarium, C. pannicola, C. pannorum, C. pruinosum, C. queenslandicum* and *C. tropicum* followed by PDA for all except *Chrysosporium georgii,* followed by CPA, CZA and MEA. Al-Musallam[5] reported that growth of mycelial biomass of *Chrysosporium zonatum* on Sabouraud's dextrose broth (SDB) and found that mycelial biomass increase exponentially to the termination of the experiment when the recorded dry weight of floating mycelial mat was 620 mg. The pH of the medium increased from 6.5 before inoculation to 8.3 at the end of experiment. Ahmed[40] investigated that nickel nanoparticles at concentrations of 50 and 100 ppm inhibited the mycelial growth of Fusarium species when grown on three different media, potato dextrose agar, corn meal agar and malt extract agar.

Conclusion

In summary, in this study, we isolated and identified *Chrysosporium queenslandicum* and analyzed various culture media and environmental conditions suitable for the rapid growth and sporulation. The information generated will facilitate mycological research on the fungus. The highest mycelium growth and sporulation on different liquid culture media, temperature and pH helps to maximize the degradation of keratinous wastes.

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References

1. Filipello M.V., Keratinophilic fungi: their role in nature and degradation of keratinic substrates. In: Kushwaha RKS, Guarro J (Eds.). Biology of Dermatophytes and other Keratinophilic Fungi. Revista Iberoamericana de Micología, Bilbao, 2000, 86-92.
2. Ruisi S., Barreca D., Selbmann L., Zucconi L., Onofri S., Fungi in Antarctica, Reviews in Environmental Science and Bio/Technology, 2007, 6(1), 127-141.

3. Sivakumar D., Gayathri G., Nishanthi R., Vijayabharathi V., Das S., Kavitha R., Role of Fungi Species in Colour Removal from Textile Industry Wastewater, International Journal of ChemTech Research, 2014, 6(9), 4366-4372.
4. Sharma A., Sharma V., Kumawat T.K., Seth R., A review on antidermatophytic efficiency of plant essential oils, Int. J. Pure App. Biosci, 2014, 2(6), 265-278.
5. Al-Musallam A.A., Al-Sane A. and Al-Zarban S.S., Nutritional controls of proteolytic and keratinolytic activities by a keratinophilic *Chrysosporium* from soil in Kuwai, Kuwait J. Sci. Eng, 2001, 28(2), 381-390.
6. Kanchana R. and Mesta D., Native Feather Degradation by a Keratinophilic Fungus, International Journal of ChemTech Research, 2013, 5(6), 2947-2954.
7. Suryanto D., Warsito K., Munir E. and Nasution S.K., Partial Characterization of Keratinase of Thermophilic Bacteria from Three Hotsprings of North Sumatra, International Journal of ChemTech Research, 2014-2015, 7(5), 2514-2519.
8. Kumawat T.K., Sharma A. and Bhadauria S., Biodegradation of Keratinous Waste Substrates by *Arthroderma multifidum*, Asian Journal of Applied Sciences, 2016, 9(3), 106-112.
9. Revathi K., Viruthagiri T. and Vimalashanmugam K., Enhanced Production of Keratinase enzyme from Chicken feathers using Response Surface Methodology, International Journal of ChemTech Research, 2015, 8(3), 1369-1381.
10. Ali A.M., El Gammal A.A. and Rifaat H.M., Influence of nutrition and culturing conditions on antimicrobial metabolite production by Streptomyces mesioensis isolated from Egyptian soil, International Journal of ChemTech Research, 2014, 6(4), 2552-2557.
11. Sharma V., Kumawat T.K., Sharma A., Seth R., Chandra S., Dermatophytes: Diagnosis of dermatophytosis and its treatment, African Journal of Microbiology Research, 2015, 9(19), 1286-1293.
12. Randhawa H.S. Sandhu R.S., A survey of soil inhabiting dermatophytes and related keratinophilic fungi of India, Sabouraudia, 1965, 4(2), 71-79.
13. Apinis A.E., Rees R.G., An undescribed keratinophilic fungus from southern Queensland, Transaction of the British Mycological Society, 1976, 67(3), 522-524.
14. Al-Musallam A., New and noteworthy fungi from Kuwait, J. Univ. Kuwait (Sci), 1987, 14, 337-350.
15. Deshmukh S.K. and Verekar S.A., Prevalence of keratinophilic fungi in public park soils of Mumbai, India, Microbiology Research, 2012, 3(e6), 24-27.
16. El-Said A.H.M., Sohair T.H. and El-Hadi A.G., Fungi associated with the hairs of goat and sheep in Libya, Mycobiology, 2009, 37(2): 82-88.
17. VissiennonT., SchuppelK.F., UllrichE. and KuijpersA.F., Case Report. A disseminated infection due to *Chrysosporium queenslandicum* in a garter snake (*Thamnophis*), Mycoses, 1999, 42(1-2), 107-110.
18. Hoshino Y., Ivanova V.B., Yazawa K., Ando A., Mikami Y., Zaki S.M., Karam A.Z., Youssef Y.A., Grafe U., Queenslandon, a new antifungal compound produced by *Chrysosporium queenslandicum*: production, isolation and structure elucidation, J. Antibiot (Tokyo), 2002, 55(5), 516-519
19. Ivanova V.B., Hoshino Y., Yazawa K., Ando A., Mikami Y., Zaki S.M., Grafe U., Isolation and structure elucidation of two new antibacterial compounds produced by *Chrysosporium queenslandicum*, J. Antibiot (Tokyo), 2002, 55(10), 914-918.
20. Sharma M. and Sharma M., Influence of environmental factors on the growth and sporulation of geophilic keratinophiles from soil samples of public park, Asian J. Exp. Sci., 2009, 23(1), 307-312.
21. Dawson C.O., Gentles J.C. and Brown E.M., Environmental conditions affecting sexual reproduction in species of *Arthroderma* and *Nannizzia*, Sabouraudia, 1964, 3(3), 245-250.
22. Reetha S., Bhuvaneswari G., Selvakumar G., Thamizhiniyan P. and Pathmavathi M. Effect of temperature and pH on growth of fungi *Trichoderma harzianum*, Journal of Chemical, Biological and Physical Sciences, 2014, 4(4), 3287-3292.
23. Abubakar A., Suberu H.A., Bello I.M., Abdulkadir R., Daudu O.A., Lateef A.A., Effect of pH on mycelial growth and sporulation of *Aspergillus parasiticus*, Journal of Plant Sciences, 2013, 1(4), 64-67.
24. Saha A., Mandal P., Dasgupta S., Saha D., Influence of culture media and environmental factors on mycelial growth and sporulation of *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl, Journal of Environmental Biology, 2008, 29(3), 407-410.
25. Zhao H., Huang L., Xiao C.L., Liu J., Wei J. and Gao X., Influence of culture media and environmental factors on mycelial growth and conidial production of *Diplocarpon mali*, Lett. Appl. Microbiol., 2010, 50(6), 639–644.

26. Vanbreuseghem R., Technique biologique pour l'isolement des dermatophytes du sol, Ann. Soc. Belge. Med. Trop., 1952, 32, 173–178.
27. Lee S.B., and Taylor J.W., Isolation of DNA from fungal mycelium and single cells, in Protocols: A Guide to Methods and Applications, Innis M.A., Gelfand H.D., Sninsky J.J. and White T.J., Eds., Academic Press, San Diego, Calif, USA, 1990, 282–287.
28. White T.J., Bruns T., Lee S. and Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, in PCR Protocols: A Guide to Methods and Applications, Innis M.A., Gelfand H.D., Sninsky J.J. and White T.J., Eds., Academic Press, San Diego, Calif, USA, 1990, 315–322.
29. Altschul S.F., Gish W., Miller W., Myers E.W., and Lipman D.J., Basic local alignment search tool, Journal of Molecular Biology, 1990, 215(3), 403-410.
30. Arey, N.C., Manual Of Environmental Analysis. Ane Books Pvt Ltd, New Delhi, India, 2010, 424.
31. Wilson M. and Knight D., Methods of plant pathology (Ed.) Tuite, John. London, Academic Press, 1952, 343.
32. Tuite J., Plant pathological Methods-Fungi and Bacteria, Burgess Publishing Company, Minneapolis, Minnesotta, 1969, 239.
33. Thiyam B. and Sharma G.D., *In Vitro* Impact of temperature on the radial growth of pathogenic fungi, Indian Journal of Applied Research,2014, 4(2), 4-5.
34. De-Maranon M.I., Chaudanson N., Joly N., Gervais P., Slow heat rate increase yeast thermotolerance by maintaining plasma membrane integrity, Biotechnol Bioeng, 1999, 65(2), 176-181.
35. Van Oorschot C.A.N., A revision of *Chrysosporium* and allied genera, Stud Mycol, 1980, 20, 1-89.
36. Ibrahim M., Rabah A.B., Liman B. and Ibrahim N.T., Effect of temperature and relative humidity on the growth of *Helminthosporium fulvum*, Nigerian Journal of Basic and Applied Science, 2011, 19(1), 127-129.
37. Kotwal S, Parate R.L., Mane S.S. and Deshmukh V.V., Influence of nutrition and different physical parameters on growth and sporulation of *Metarhizium anisopliae*, International Journal of Science, Environment and Technology, 2012, 1(5), 479-484.
38. Gupta S.L., Rizvi G., Paijwar M.S., Effects of different culture media on colony growth of keratinophilic and non-keratinophilic fungi, Trends in Biosciences, 2013, 6(5), 637-640.
39. Verma V.S., Effect of temperature and hydrogen ion concentration on three pathogenie Fungi, Sydowia, 1969, 23, 164-168.
40. Ahmed A.I.S., Yadav D.R. and Lee Y.S., In Vitro Evaluation of Nickel Nanoparticles against Various Pathogenic Fusarium Species, International Journal of ChemTech Research, 2016, 9(6), 174-183.

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