Scientific Research Publishing

American Journal of Plant Sciences, 2022, 13, 506-516
https://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

First Report on Rhizome Rot Disease of *Curcuma longa* Caused by *Fusarium solani* in Bangladesh

Md. Sabbir Ahmmed, Fatema Akter Nisha, Nuhu Alam*

Department of Botany, Faculty of Biological Sciences, Jahangirnagar University, Dhaka, Bangladesh
Email: *mnabotju@yahoo.com

How to cite this paper: Ahmmed, Md.S., Nisha, F.A. and Alam, N. (2022) First Report on Rhizome Rot Disease of *Curcuma longa* Caused by *Fusarium solani* in Bangladesh. *American Journal of Plant Sciences*, **13**, 506-516.
https://doi.org/10.4236/ajps.2022.134033

Received: March 7, 2022
Accepted: April 25, 2022
Published: April 28, 2022

Abstract

Turmeric (*Curcuma longa* L.) is a valuable medicinal plant as well as spice crop in Bangladesh. The rhizome rot disease is a severe danger to turmeric cultivation. The current study sought to identify the fungal pathogen linked to turmeric rhizome rot disease. Rhizome of turmeric with distinct rotted symptoms was collected from the experimental site of the Botanical Garden, Jahangirnagar University, Bangladesh. The sample was screened to isolate the causative fungal pathogen through the tissue planting technique. Macro and micro-morphological characterization based on colony appearance, mycelial and conidial characteristics primarily identified the fungus as *Fusarium* sp. The ITS sequence of rDNA of the fungus exhibited 99 to 100 percent similarity with the other *F. solani* species formerly deposited in the NCBI database which confirmed the fungal identity as *F. solani*. An *in vitro* pathogenicity test validated the pathogenic nature of the fungus. Growth behaviors of the fungus were evaluated on different solid culture media *viz.*, Potato dextrose agar, Potato sucrose agar, Sabouraud dextrose agar and Hansen's agar; temperature conditions (10˚C, 15˚C, 20˚C, 25˚C, 30˚C and 35˚C) and pH levels (pH 4, pH 5, pH 6, pH 7 and pH 8). Maximum mycelial growth was obtained on PSA medium at 30˚C temperature and pH 7 conditions. Current findings also conclude that *F. solani* favors a wide range of temperature and pH levels. To the best of our search, the present investigation revealed the relationship of *F. solani* with the rhizome rot disease of turmeric for the first time in Bangladesh.

Keywords

Turmeric, Fungal Disease, Molecular Identification, Culture Media, Temperature, pH

1. Introduction

Turmeric *(Curcuma longa* L.) is a perennial, cylindrical, cross-pollinated rhizomatous herb belonging to the family Zingiberaceae [1] [2]. It has about 40 different genera and 400 species and the most common varieties are *Curcuma longa, Curcuma aromatic, Curcuma amada, Curcuma algustifolia, Curcuma zidoria.* Turmeric is distributed throughout the subtropical and tropical regions of the world, such as India, South-East Asian countries, and North Australia [3]. India is the world's biggest producer of turmeric, accounting for 75 - 80 percent of global output, followed by China, Myanmar, Nigeria, and Bangladesh [4]. In Bangladesh, Turmeric cultivation is a promising source of livelihood for hilly people and about 150 turmeric farms are located in the Khagrachari district of Bangladesh [5].

Curcumin, the active ingredient, is thought to have antimicrobial and anti-inflammatory properties and appears as an effective cure for several diseases [6] [7] [8]. *C. longa* contains carbohydrates (69.4%), minerals (3.5%), protein (6.3%), fat (5.1%), fiber (2.6%), and moisture (13.2%) [9]. It has an extremely important role in Hindu and Buddhist culture. It is also an essential spice and has become an inseparable part of some special dishes. For its wonderful dietary properties and acrimonious taste, this plant is referred to as the "Queen of Spices". The Bangladeshi people usually use turmeric in all curry preparation like fish, meat, vegetables, pulse, etc. for its typical color and bitter flavor [5]. Dried rhizomes of turmeric are used as condiments, dye, drugs, and cosmetics. It is also said to cleanse the liver, lower cholesterol, treat allergies, improve digestion, promote immunity, and improve appearance [10]. *Curcuma* sp. has been used as a medicinal herb due to its diverse superior characteristics such as antioxidant [11], anti-inflammatory [12], antimicrobial [13] and several therapeutic properties [14].

Although having so many therapeutic and antimicrobial properties turmeric is vulnerable to several fungal pathogens of both soil and air borne nature that reduce the quality as well as quantity of the crop production. The incidence of rhizome rot disease was surveyed and the causal organism of diseases was isolated in major turmeric growing tracts of South India [10]. In India, *Fusarium solani* and *Pythium aphanidermatum* have been reported to cause rhizome rot disease of turmeric [15] [16] [17] [18] [19]. The occurrence of rhizome rot disease of turmeric caused by *Pythium aphanidermatum* (Edson) Fitzp. has been recorded in Karnatak, Kerala, Tamilnadu, Andhra Pradesh states of India [6]. Another study has also been conducted in Tamil Nadu, India, and found the association of *Fusarium solani* and some other pathogens to cause rhizome rot infection [20]. In Bangladesh, numerous endophytic fungi were reviewed to associate with the rhizome of turmeric [21]. But there is still no published article on the rhizome rot disease of turmeric in Bangladesh. Hence, the present investigation was designed to detect and identify the causal fungal agent of rhizome rot disease of turmeric through morphological and molecular characterization and to study the effect of different physical factors (media, temperature and pH) on

the growth pattern of the fungal pathogen.

2. Materials and Methods

2.1. Isolation and Identification of the Pathogen

Infected rhizome of turmeric with characteristic symptoms was collected in a sterilized zipper polybag from the experimental site of the Botanical Garden, Jahangirnagar University, Savar, Dhaka, Bangladesh. The diseased sample was washed with distilled water followed by surface sterilization with 5% NaOCl for 3 minutes. The sample was then rinsed with distilled water 3 - 4 times and subsequently air-dried into the laminar airflow cabinet until the surface water was disappeared. The diseased sample was cut into pieces and inoculated into a Potato dextrose agar (PDA) medium following the tissue planting method and incubated at room temperature (25˚C ± 2˚C) to isolate the fungal pathogen associated with the rhizome rot disease [22].

The isolated fungal pathogen was identified through morphological and molecular techniques. Morphological identification was carried out by observing macro and microscopic characteristics of the colony, mycelial and conidia such as colony appearance, mycelial color, branching pattern of mycelium, shape, size and pigmentation of the conidia etc. In the molecular approach, the fungal isolate was identified using the internal transcribed spacer (ITS) sequence of the rDNA region. DNA was extracted using a DNA extraction kit (Promega, USA). PCR amplification of the target region of DNA was performed using ITS4 and ITS5 primers [23]. The PCR cycles were performed with Taq polymerase activation at 94˚C for 5 minutes, 35 cycles of 94˚C for 30 seconds, 55˚C for 30 seconds, and 72˚C for 5 minutes followed by a 10-minute step at 72˚C [24]. The Maxwell® 16 LEV Plant DNA Kit (Promega, USA) was used to purify the PCR product. The PCR product was sent to First BASE Laboratories (Sdn Bhd, Malaysia) for sequencing. The sequenced data were then submitted to NCBI to assign an accession number to the work.

2.2. Pathogenicity Test

A healthy rhizome of turmeric was detached from the susceptible cultivar and surface sterilized with 5% NaOCl and then cleaned with distilled water after being washed with flowing tap water. The artificial wound was made by using a sterilized needle and inoculated with a 2 mm diameter agar block containing tested fungal pathogen [25]. Artificially inoculated rhizome was kept at 25˚C ± 2˚C in a desiccator with moistened cotton to preserve humidity. After 35 - 40 days of incubation, a typical symptom was reproduced and the pathogen was re-isolated from the artificially inoculated plant part to corroborate Koch's postulates.

2.3. Growth Response of the Fungus to Culture Media, Temperature and pH

Culture media, temperature and pH play a vital role in fungal growth and de-

velopment. Four different fungal culture media, Potato sucrose agar (PSA), Sabouraud dextrose agar (SDA), Potato dextrose agar (PDA), and Hansen's agar (HA); six different temperature regimes (10˚C, 15˚C, 20˚C, 25˚C, 30˚C, and 35˚C) and five different pH levels (4.0, 5.0, 6.0, 7.0, and 8.0) were employed and observed to evaluate the effects on the growth and development of the fungus. A 2 mm diameter agar plug was removed from 7 days old culture of the pathogen grown on PDA medium and inoculated on the center of each plate containing culture medium. The effects of temperature and pH were observed on the PDA medium. The experiment was conducted in controlled environmental conditions and mycelial growth was recorded at 7 days post-incubation (dpi) [24] [26].

2.4. Statistical Analysis

Statistical analysis of the data generated during the experiments was analyzed using standard statistical analyzing tools *viz*; MS Excel, SPSS 16.0, MEGA 6 software and BLAST tool, etc.

3. Result and Discussion

3.1. Identification of the Fungus

Rhizome rot disease symptom of turmeric was observed light brown color in the margin with deep brown rotted center. The diseased tissues were inoculated into the Potato dextrose agar (PDA) medium and found a cottony white-colored fungal colony after 3 days of incubation. The upper surface of the colony was white, wooly with a slightly elevated center and the lower surface of the colony was off-white color. Under the compound microscope, the mycelium of the fungus was found hyaline, septate, and branched. Macroconidia were hyaline, 2 - 4 celled with thick wall, slightly curved, and blunt-ended. Microconidia are abundant and oval to kidney-shaped. Chlamydospores were also formed in the fungal mycelia (**Figure 1**). *Fusarium solani* (Mart.) Sacc causing wilt disease of bitter gourd in India was identified through cultural and morphological characterization [27].

The molecular identification of the fungus was carried out by analyzing the ITS sequence of rDNA region of the fungus. Data were submitted to NCBI and received an accession number OL691079.1. The sequenced data were compared with the database previously deposited to NCBI through BLAST search. Our organism OL691079.1 showed 100% similarity with *Fusarium solani* (KY949606.1 and MN653247.1) followed by above 99% similarity with *Fusarium solani* (MK-174970.1, KY013237.1, OL314780.1 and MK174971.1). The phylogenetic tree was produced to reveal the closest matching taxa. This analysis involved 30 nucleotide sequences. In the maximum likelihood tree (**Figure 2**), current studied fungus (OL691079.1) form cluster with other species of *Fusarium solani* which revealed that the fungus is *Fusarium solani*. *F. solani* species complex were also identified through phylogenetic analysis in Malaysia [28].

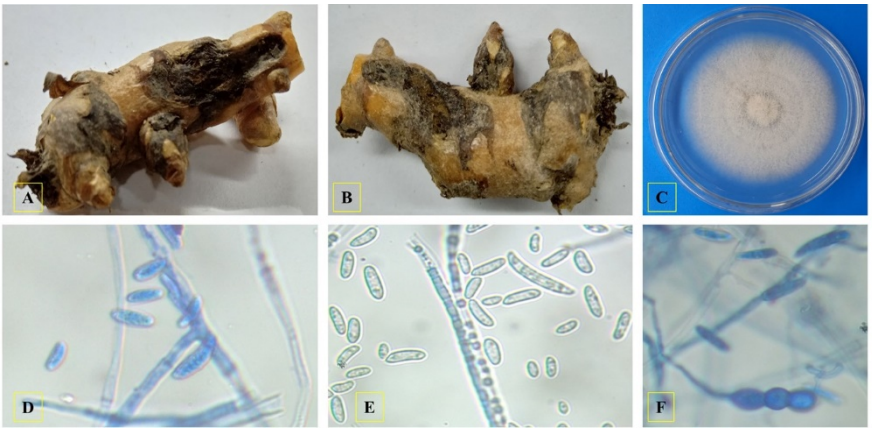

Figure 1. Morphological characterization of *Fusarium solani* causing rhizome rot disease of Turmeric (A—Rhizome rot symptom in the primary stage; B—Symptom in advanced stage; C—Mycelial growth of the fungus on PDA medium; D—Branched, septate mycelia with conidia (40X); E—Macro and microconidia (40X); F—Chlamydospore).

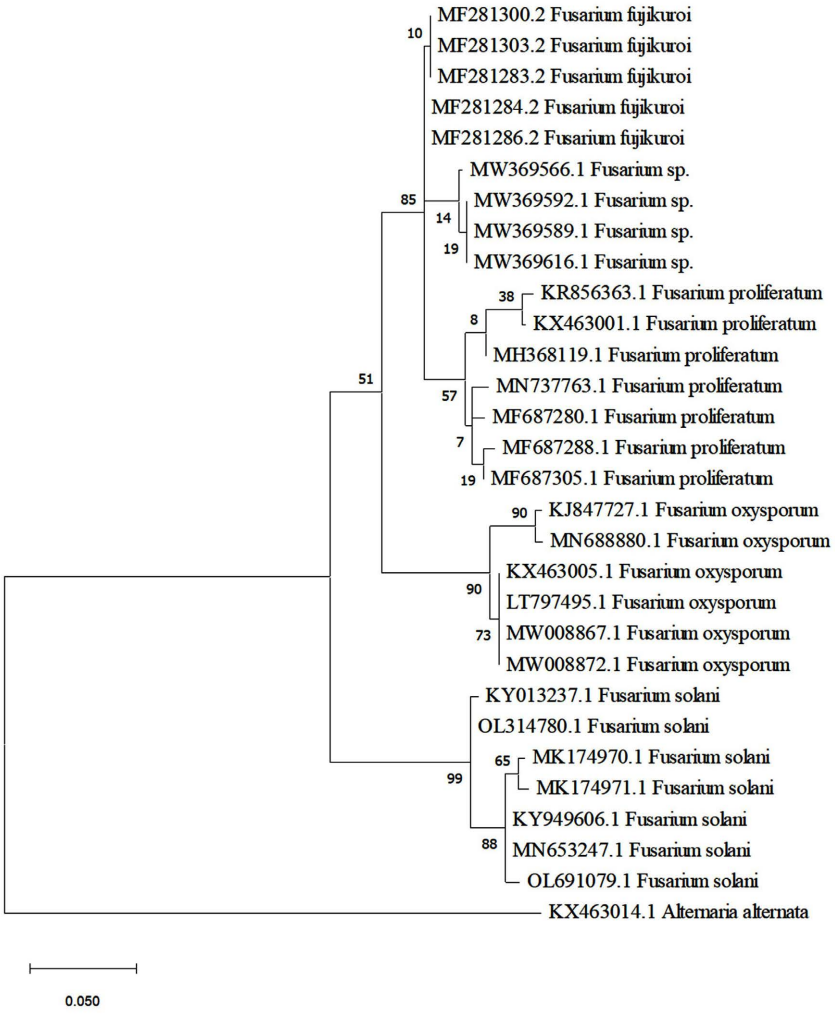

Figure 2. Maximum Likelihood (ML) tree derived from analysis of ITS sequence dataset of the studied fungus with bootstrap value (Bootstrap replication = 1000). The accession number of our organism is OL691079.1.

3.2. Pathogenicity Test

The healthy susceptible rhizome of turmeric (*C. longa*) was artificially inoculated with respective fungal isolate under *in vitro* condition in a desiccator following the modified "detached leaf technique" (**Figure 3**). Typical symptom with a light brown margin and dark brown center was reproduced after 35 - 40 days of inoculation (**Figure 3**). The fungus was reisolated and characterized and found identical with the primarily isolated organism which proved the pathogenic nature of *Fusarium solani* causing rhizome rot disease of turmeric (**Figure 3**). An experiment has been conducted to test the pathogenicity of *Fusarium langsethiae* towards oats and wheat in *in vitro* conditions using the detached leaf technique in the UK [29].

3.3. Growth Rresponse of *Fusarium solani* to Culture Media

The growth and development of fungi are largely dependent on the media where they grow. In the current investigation, four different solid culture media such as PDA, SDA, PSA and HA were used and the results revealed that the highest mycelial growth of *F. solani* was obtained on PSA medium followed by PDA, SDA and HA at 7 days post-incubation (dpi) in room temperature (25˚C ± 2˚C) (**Figure 4**). These findings showed analogies with the previous study. Mean mycelial growth of *Fusarium* spp. (47.58 mm) and *F. solani* (61.94 mm) was observed on PDA and PSA media respectively [30]. The best growth of *F. solani* was obtained on the Sabouraud dextrose agar (SDA) medium [31]. In India, the influence of physiological variables on the growth of *Fusarium* sp was studied using eight different solid culture media *viz.,* Corn meal agar (CMA), Potato carrot agar (PCA), Potato dextrose agar (PDA), Richards' agar (RA), Host extract agar (HEA), Oatmeal agar (OMA), Sabouraud maltose agar (SMA) and V-8 juice agar (V8JA) to identify the best medium for growth and development of *F. solani* and highest radial growth was found on PDA medium (90 mm) and lowest on RA medium (63 mm) [32].

3.4. Growth Response of *F. solani* to Temperature

During the present investigation, six different temperature regimes were tested *viz.*, 10˚C, 15˚C, 20˚C, 25˚C, 30˚C, and 35˚C on PDA medium. The highest mycelial growth of *F. solani* was found at 30˚C followed by 25˚C and 35˚C. The mycelial growth almost ceased at 10˚C (**Figure 5**). According to the data, *F. solani* prefers a temperature range of 25˚C - 35˚C for normal growth and development, however, at low temperatures, its growth is halted. Previous experiments showed similarities with the current result. In India, the growth characteristics of *Fusarium* spp. were studied and 28˚C was recorded as the optimum temperature for the growth and development of *F. solani* [33]. The effect of temperature on *F. solani* causing disease in soybean was investigated and 29.3˚C was estimated as the optimum temperature for the proliferation of the fungal pathogen [34]. *F. solani* revealed the highest growth performance at 25˚C [31].

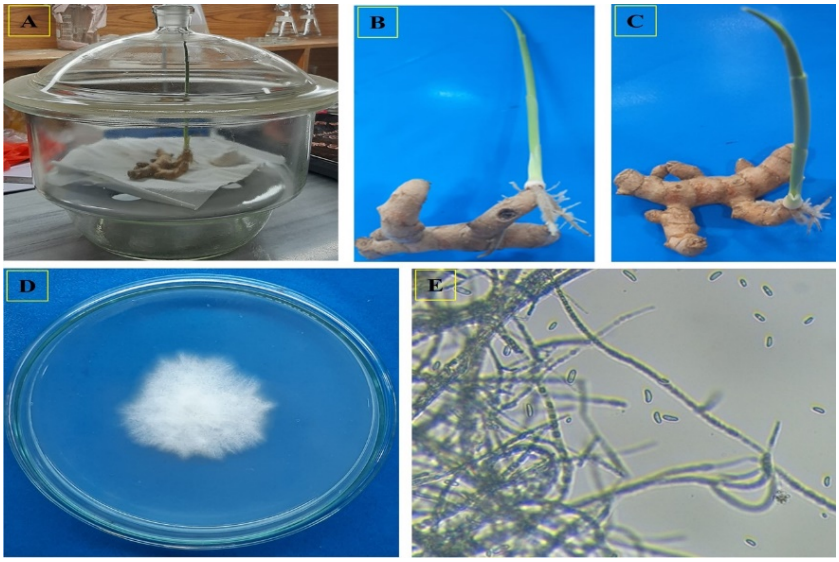

Figure 3. Pathogenicity test in *in vitro* condition (A—Experiment set up in desiccator; B—Reproduced rot symptom; C—Control; D—Re-isolated fungal pathogen on PDA medium; E—Microscopic view of *F. solani* (10X)).

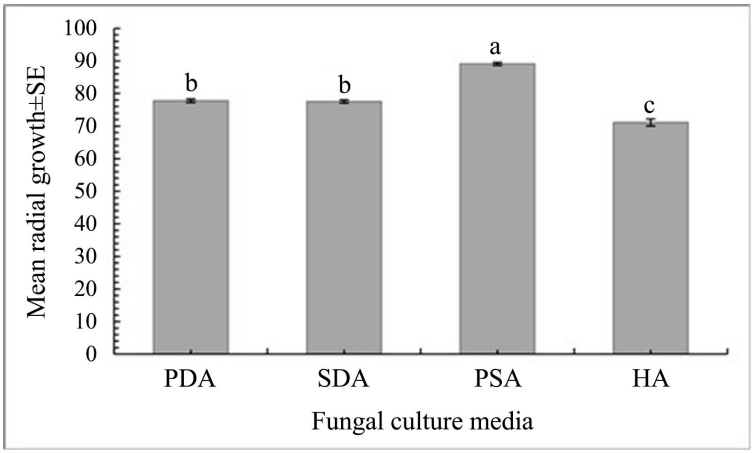

Figure 4. Effect of culture media on the mycelial growth of *F. solani* at 7 dpi.

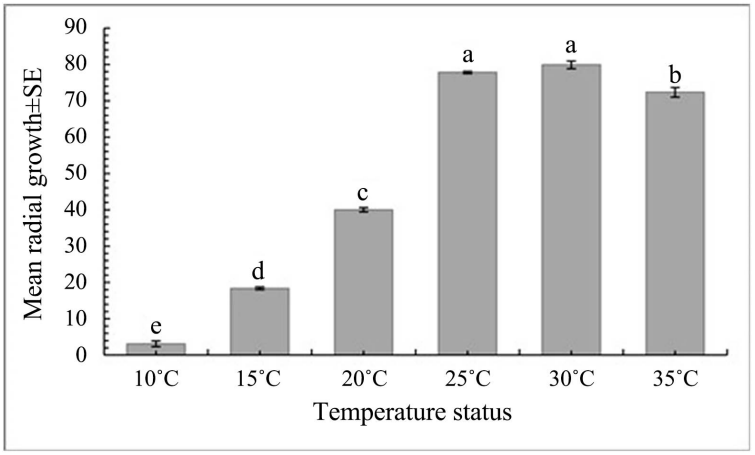

Figure 5. Effect of temperature on the mycelial growth of *F. solani* at 7 dpi.

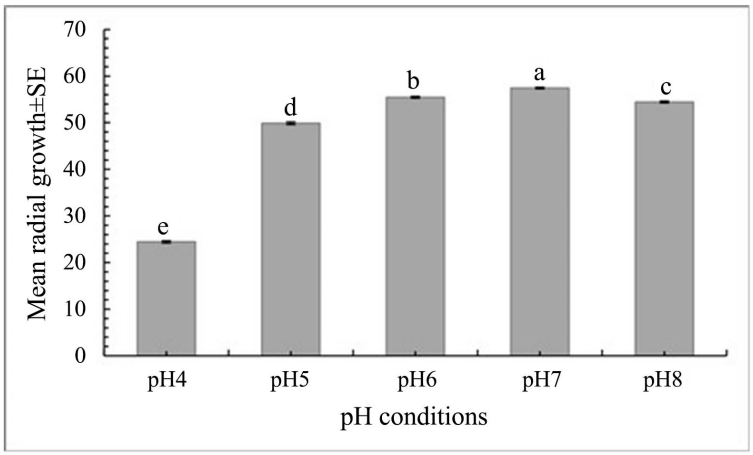

Figure 6. Effect of pH on the mycelial growth of *F. solani* at 7 dpi.

3.5. Growth Response of *F. solani* to pH

Five several pH levels of culture media *viz.*, 4.0, 5.0, 6.0, 7.0, and 8.0 were tested in the running investigation and the highest radial mycelial growth of *F. solani* was recorded at pH 7.0 and the lowest growth was recorded at pH 4.0 after 7 days of incubation (dpi) (**Figure 6**). Almost similar results were found at pH 6.0, 7.0 & 8.0, which indicate that *F. solani* favours a wide range of pH conditions. These findings are very comparable to antecedent works. The highest mycelial growth of *F. solani* occurred at pH 6.0, followed by pH 7.0, which is quite analogous to the current study [32]. The growth and bioactive metabolite production of *Fusarium solani* were optimized in India and pH 6.0 was noticed as the optimum condition for the proliferation of the fungus [35]. Optimum pH of 7.5 is favorable for the growth of all species of *Fusarium* [31].

Acknowledgements

This research work was funded by Jahangirnagar University research project grand for the fiscal year 2020-2021.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Damalas, C.A. (2011) Potential Uses of Turmeric ('*Curcuma longa*') Products as Alternative Means of Pest Management in Crop Production. *Plant OMICS*, **4**, 136-141.

[2] Gupta, A.K., Mishra, R. and Lal, R.K. (2015) Genetic Resources, Diversity, Characterization and Utilization of Agronomical Traits in Turmeric (*Curcuma longa* L.). *Industrial Crops and Products*, **77**, 708-712. https://doi.org/10.1016/j.indcrop.2015.09.030

[3] Mirjanaik, R.H. and Vishwanath, Y.C. (2020) Advances in Production Technology of Turmeric. *Journal of Pharmacognosy and Phytochemistry*, **9**, 1198-1203.

[4] Vinayarani, G. and Prakash, H.S. (2018) Growth Promoting Rhizospheric and Endophytic Bacteria from *Curcuma longa* L. as Biocontrol Agents against Rhizome Rot and Leaf Blight Diseases. *The Plant Pathology Journal*, **34**, 218-235. https://doi.org/10.5423/PPJ.OA.11.2017.0225

[5] Begum, M.E.A., Miah, M.M., Rashid, M.A., Islam, M.T. and Hossain, M.I. (2019) Economic Analysis of Turmeric Cultivation: Evidence from Khagrachari District. *Bangladesh Journal of Agricultural Research*, **44**, 43-58. https://doi.org/10.3329/bjar.v44i1.40902

[6] Vinayarani, G. and Prakash, H.S. (2018) Fungal Endophytes of Turmeric (*Curcuma longa* L.) and Their Biocontrol Potential against pathogens *Pythium aphanidermatum* and *Rhizoctonia solani*. *World Journal of Microbiology and Biotechnology*, **34**, Article No. 49. https://doi.org/10.1007/s11274-018-2431-x

[7] Chen, D.Y., Shien, J.H., Tiley, L., Chiou, S.S., Wang, S.Y., Chang, T.J., Lee, Y.J., Chan, K.W. and Hsu, W.L. (2010) Curcumin Inhibits Influenza Virus Infection and Haemagglutination Activity. *Food Chemistry*, **119**, 1346-1351. https://doi.org/10.1016/j.foodchem.2009.09.011

[8] Koosirirat, C., Linpisarn, S., Changsom, D., Chawansuntati, K. and Wipasa, J. (2010) Investigation of the Anti-Inflammatory Effect of *Curcuma longa* in *Helicobacter Pylori*-Infected Patients. *International Immunopharmacology*, **10**, 815-818. https://doi.org/10.1016/j.intimp.2010.04.021

[9] Trinidad, P.T., Sagum, R.S., de Leon, M.P., Mallillin, A.C. and Borlagdan, M.P. (2012) *Zingiber officinale* and *Curcuma longa* as Potential Functional Foods/Ingredients. *Food and Public Health*, **2**, 1-4. https://doi.org/10.5923/j.fph.20120202.01

[10] Anoop, K. and Suseela Bhai, R. (2013) Host Range Study of Turmeric Rhizome Rot Pathogen *Pythium aphanidermatum* on Selected Zingiberaceae Members. *International Journal of Research in Pure and Applied Microbiology*, **3**, 113-115.

[11] Abdel Rheim, F., Ragab, A.A., Hammam, F. and Hamdy, H.E.D. (2015) Evaluation of DNA Damage *in Vivo* by Comet Assay and Chromosomal Aberrations for Pyrethroid Insecticide and the Antimutagenic Role of Curcumin. *The Egyptian Journal of Hospital Medicine*, **59**, 172-181. https://doi.org/10.12816/0012174

[12] Gómez-Estaca, J., Balaguer, M.P., López-Carballo, G., Gavara, R. and Hernández-Muñoz, P. (2017) Improving Antioxidant and Antimicrobial Properties of Curcumin by Means of Encapsulation in Gelatin through Electrohydrodynamic Atomization. *Food Hydrocolloids*, **70**, 313-320. https://doi.org/10.1016/j.foodhyd.2017.04.019

[13] Prasad, S., Tyagi, A.K. and Aggarwal, B.B. (2014) Recent Developments in Delivery, Bioavailability, Absorption and Metabolism of Curcumin: The Golden Pigment from Golden Spice. *Cancer Research and Treatment: Official Journal of Korean Cancer Association*, **46**, 2-18. https://doi.org/10.4143/crt.2014.46.1.2

[14] Gupta, S.C., Patchva, S. and Aggarwal, B.B. (2013) Therapeutic Roles of Curcumin: Lessons Learned from Clinical Trials. *The AAPS Journal*, **15**, 195-218. https://doi.org/10.1208/s12248-012-9432-8

[15] Reddy, M.N., Devi, M.C. and Sreedevi, N.V. (2003) Biological Control of Rhizome Rot of Turmeric (*Curcuma longa* L.) Caused by *Fusarium solani*. *Journal of Biological Control*, **17**,193-195.

[16] Anusuya, S. and Sathiyabama, M. (2014) Effect of Chitosan on Rhizome Rot Disease of Turmeric Caused by *Pythium aphanidermatum*. *International Scholarly Research Notices*, **2014**, Article ID: 305349. https://doi.org/10.1155/2014/305349

[17] Sarathi, V., Senthil, K., Kumar, R.S. and Panneerselvam, A. (2014) Studies on Rhi-

zome Rot Pathogen in *Curcuma longa*. *International Journal of Current Microbiology and Applied Sciences*, **3**, 296-302.

[18] Anoop, K., Suseela Bhai, R. and Shiva, K.N. (2014) A Survey on the Incidence of Rhizome Rot Disease in Major Turmeric Growing Tracts of South India and Isolation of Associated Organisms. *Indian Journal of Advances in Plant Research*, **1**, 17-23. https://www.researchgate.net/publication/268503749

[19] Chavan, P.G., Apet, K.T. and Borade, R.S. (2017) Integrated Management of Turmeric Rhizome Rot Caused by *Pythium aphanidermatum*. *International Journal of Current Microbiology and Applied Sciences*, **6**, 5321-5327. https://doi.org/10.20546/ijcmas.2017.611.508

[20] Chenniappan, C., Daniel, G.M., Ponnusamy, P. and Narayanasamy, M. (2020) Association of Multiple Fungal Pathogens to Rhizome Rot Disease of Turmeric. *Archives of Phytopathology and Plant Protection*, **53**, 440-458. https://doi.org/10.1080/03235408.2020.1749428

[21] Ferdous, K.J., Afroz, F., Islam, M.R., Mazid, M.A. and Sohrab, M.H. (2019) Isolated Endophytic Fungi from the Plant *Curcuma longa* and Their Potential Bioactivity—A Review. *Pharmacology & Pharmacy*, **10**, 244-270. https://doi.org/10.4236/pp.2019.105021

[22] Ahmmed, M.S., Sikder, M.M., Sultana, A., Sultana, S. and Alam, N. (2020) First Report on Leaf Spot Disease of *Aloe vera* Caused by *Alternaria alternata* (Fr.) Keissler in Bangladesh. *International Journal of Botany Studies*, **5**, 164-169. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

[23] White, T.J., Bruns, T., Lee, S.J.W.T. and Taylor, J. (1990) Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. *PCR Protocols*: *A Guide to Methods and Applications*, **18**, 315-322.

[24] Sikder, M.M., Mallik, M.R.I. and Alam, N. (2019) Identification and *in Vitro* Growth Characteristics of Entomopathogenic Fungus-*Aschersonia* sp. in Bangladesh. *Advances in Zoology and Botany*, **7**, 11-18. https://doi.org/10.13189/azb.2019.070102

[25] Singh, A., Sohi, G.S. and Singh, B. (2020) Pathogenicity Test by Using Artificial Inoculation Methods and Eco-Friendly Management of *Albugo candida* on *Brassica juncea* under Punjab Region. *Journal of Plant Development Sciences*, **12**, 153-158.

[26] Ahmmed, M.S., Sikder, M.M., Sultana, A., Sultana, S., and Alam, N. (2021). First Report of Leaf Spot Disease of *Aloe vera* Caused by *Fusarium oxyporum* in Bangladesh. *International Journal of Progressive Sciences and Technologies*, **29**, 251-260.

[27] Manohari, J., Latha, P., Kamalakannan, A., Selvakumar, S. and Karthikeyan, M. (2020) Characterization of *Fusarium solani* (Mart.) Sacc. Causing Fusarium Wilt of Bitter Gourd in Coimbatore Region. *International Journal of Current Microbiology and Applied Sciences*, **9**, 2336-2349. https://doi.org/10.20546/ijcmas.2020.906.286

[28] Chehri, K., Salleh, B. and Zakaria, L. (2015) Morphological and Phylogenetic Analysis of *Fusarium solani* Species Complex in Malaysia. *Microbial Ecology*, **69**, 457-471. https://doi.org/10.1007/s00248-014-0494-2

[29] Imathiu, S.M., Ray, R.V., Back, M., Hare, M.C. and Edwards, S.G. (2009) *Fusarium langsethiae* Pathogenicity and Aggressiveness towards Oats and Wheat in Wounded and Unwounded *in Vitro* Detached Leaf Assays. *European Journal of Plant Pathology*, **124**, 117-126. https://doi.org/10.1007/s10658-008-9398-7

[30] Mezzomo, R., Rolim, J.M., Poletto, T., De Oliveira, M.B., Lazarotto, M. and Muñiz, M. (2018) Mycelial Growth and Sporulation of *Fusarium* spp. Pathogenic to *Ilex paraguariensis* in Different Culture Media and under Exposure to Different Light Levels. *Scientia Agraria*, **19**, 14-19. https://doi.org/10.5380/rsa.v19i1.55844

[31] Mohsen, L., Al-Janabi, J.K. and Jebor, M.A. (2016) The Effect of Some Environmental Conditions on the Growth and Activity of the External Enzymes for Five sp. of *Fusarium*. *Journal of Babylon University/Pure and Applied Sciences*, **24**, 630-646.

[32] Ramesha, V., Naik, K. and Raj, D. (2019) Effect of Physiological Parameters on the Growth of *Fusarium* sp. *The Pharma Innovation Journal*, **8**, 492-495.

[33] Gupta, V., Misra, A. and Gaur, R. (2010) Growth Characteristics of *Fusarium* spp. Causing Wilt Disease in *Psidium guajava* L. in India. *Journal of Plant Protection Research*, **50**, 452-462. https://doi.org/10.2478/v10045-010-0076-3

[34] Yan, H. and Nelson Jr., B. (2020) Effect of Temperature on *Fusarium solani* and *F. tricinctum* Growth and Disease Development in Soybean. *Canadian Journal of Plant Pathology*, **42**, 527-537. https://doi.org/10.1080/07060661.2020.1745893

[35] Merlin, J.N., Christhudas, I.V.S.N., Kumar, P.P. and Agastian, P. (2013) Optimization of Growth and Bioactive Metabolite Production: *Fusarium solani*. *Asian Journal of Pharmaceuticals and Clinical Research*, **6**, 98-103.