AgEcon SEARCH
RESEARCH IN AGRICULTURAL & APPLIED ECONOMICS

The World's Largest Open Access Agricultural & Applied Economics Digital Library

This document is discoverable and free to researchers across the globe due to the work of AgEcon Search.

Help ensure our sustainability.

Give to AgEcon Search

AgEcon Search
http://ageconsearch.umn.edu
aesearch@umn.edu

*Papers downloaded from **AgEcon Search** may be used for non-commercial purposes and personal study only. No other use, including posting to another Internet site, is permitted without permission from the copyright owner (not AgEcon Search), or as allowed under the provisions of Fair Use, U.S. Copyright Act, Title 17 U.S.C.*

No endorsement of AgEcon Search or its fundraising activities by the author(s) of the following work or their employer(s) is intended or implied.

ISSN: 2224-0616
Int. J. Agril. Res. Innov. Tech. 11(2): 124-132, Dec 2021
DOI: https://doi.org/10.3329/ijarit.v11i2.57265
OPEN ACCESS
Available online at https://ijarit.webs.com
https://www.banglajol.info/index.php/IJARIT

Molecular characterization and vegetative growth of pathogenic seed-borne fungus, *Curvularia lunata* of tomato and its *in vitro* control measures

M.B. Billah, M.M. Sikder, M.R.I. Mallik, M.K. Hossain and N. Alam*

Received 11 September 2021, Revised 16 November 2021, Accepted 20 December 2021, Published online 31 December 2021

ABSTRACT

Present studies were conducted to isolate and identify the seed-borne pathogenic fungus from the selected tomato variety through morphological and molecular techniques based on the sequencing of internal transcribed spacer (ITS) region of 18S rDNA. According to the colony and conidial features, the fungus was identified as *Curvularia* sp. The obtained ITS sequencing showed above 99% similarity with *Curvularia lunata* in the NCBI database. The sequence of the fungus was deposited in NCBI GenBank under the accession number: ITS, MH382879.1. Besides, the phylogenetic tree further confirmed the taxonomic position of the studied fungus. Growth characteristics of the fungus on nine different fungal culture media were evaluated, in which Honey peptone agar, Carrot agar, Potato sucrose agar, and Kauffman's agar were found the most suitable. The maximum vegetative growth of the fungus was recorded at 30°C temperature and pH conditions. The bio-control potential of five different antagonists against the studied fungus was assessed, in which *Trichoderma harzianum* showed the better performance to restrict mycelial growth. Three ethanolic plant extracts were also evaluated, in which *Lowsonia inermis* L. exhibited above 60% mycelial growth inhibition of the fungus. Among three tested fungicides, Tilt 250 EC was found as an excellent fungicide to inhibit mycelial growth of *C. lunata* under *in vitro* conditions.

Keywords: Tomato, Fungal biology, Bio-control agents, Fungicides, Bangladesh

Laboratory of Mycology and Plant Pathology, Department of Botany, Faculty of Biological Sciences, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh

*Corresponding author's email: mnabotju@yahoo.com (N. Alam)

Cite this article as: Billah, M.B., Sikder, M.M., Mallik, M.R.I., Hossain, M.K. and Alam, N. 2021. Molecular characterization and vegetative growth of pathogenic seed-borne fungus *Curvularia lunata* of tomato and its *in vitro* control measures. *Int. J. Agril. Res. Innov. Tech.* 11(2): 124-132. https://doi.org/10.3329/ijarit.v11i2.57265

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is a popular vegetable crop belonging to the family Solanaceae. It is especially honoured due to its high nutritive value, taste, and versatile use as vegetable, food, and preparation of various food items. It is widely grown in almost all countries of the world due to its adaptability to a wide range of soils and climatic conditions. The quality of seed plays an important role in crop production. However, seeds are known to be vulnerable to attack by seed-borne fungi either saprophytically or within the tissues of the embryo (Vijendra and Sinclair, 1997). Infected seeds play an important role in the spreading of plant pathogens and disease establishment (Agarwal, 1981). The disease-causing fungal pathogens might be involved in shrunken seed, seed abortion, seed rot, seed discoloration, and causes of reduction of germination capacity (Hamim *et al.*, 2014). *Curvularia* spp. are internally and externally seed-borne pathogenic fungi, affecting seed germination and seedling vigour, known to be responsible for pre-and post-emergence seedling mortality (Gupta *et al.*, 2017). They cause seed disclouration, even produce toxins that may be injurious to man and domestic animals.

Seed treatment is the safest and the cheapest way to control seed-borne fungal diseases and to prevent bio-deterioration of grains (Bagga and Sharma, 2006). However, uses of fungicides are not considered as sustainable solutions due to their harmful effects on human beings as well as soil health. Therefore, nowadays the focus is shifting in the direction of exploitation of biological organisms and natural products for the control of plant diseases as an alternative way to synthetic fungicides. Environmental factors such as temperature, pH, and water activity play a key role in fungal development (Yadav *et al.*, 2014). Carbon and nitrogen sources available in the fungal culture media besides changes in environmental factors, incubation time, and shaking have a significance influence on the growth of microbial pathogen (Tyagi and Paudel, 2014). Hence, the present studies were

An open access article under CC BY

undertaken to isolate and identify of seed-borne tomato fungal pathogen using morphological and molecular techniques; to study growth characteristics of the fungus; to evaluate the efficacy of biological agents, plant extracts, and chemical fungicides against the isolated fungus.

Methodology

Pathogen isolation and identification

For isolation of the fungal pathogen from tomato seeds of selected varieties, a direct isolation method was performed. This method consisted of incubation of surface sterilized tomato seeds in a humid chamber following the paper towel method. For the identification of isolated fungus, we used a standard manual-Dematiaceous hyphomycetes by Ellis (1971), and further confirmation was done via molecular techniques.

Molecular characterization

Fungus genomic DNA samples were extracted using Maxwell Cell Kit (Promega, Madison, USA). The primers ITS-4 primer (5′-TCCTCCGCTTATTGATATGC-3′) and ITS-5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) was used to amplify the target region of the fungus (White *et al.*, 1990). The PCR reaction was performed in a 25 µl reaction mixture, consisting of 5 µl DNA (20ng/µl) template, 12.5 µl GoTaq G2 Hot Start Green Master Mix (dNTPs, Buffer, $MgCl_2$, Taq Polymerase; Promega 2X, Promega, Madison, USA), 2.5 µl of each primer (10 µM), and 2.5 µl water. The PCR reaction was performed with the activation of Taq polymerase at 94°C for 60 Sec, 35 cycles of 94°C for 30 Sec, 55°C for 30 Sec, 72°C for 5 minutes, and termination at 72°C for 10 minutes (Sikder *et al.*, 2019).

The Maxwell® 16 DNA Purification Kit was used to purify the PCR products (Promega, USA). The purified PCR product of approximately 650 bp was sequenced in First BASE Laboratories Sdn Bhd (Malaysia). Sequencing data was blastn searched and compared with similar DNA sequences after retrieving from NCBI Genbank. The phylogenetic analysis was conducted by the multiple sequence alignment tools using MEGA 6 software (Tamura *et al.*, 2013).

Effect of fungal culture media, temperature, and pH on the mycelial growth of Curvularia lunata

Nine different culture media i.e. Potato Dextrose Agar (PDA), Yeast Extract Agar (YEA), Honey Peptone Agar (HPA), Hansen's Agar (HA), Sobouraud's Glucose Agar (SGA), Kauffman's Agar (KA), Potato Sucrose Agar (PSA), Richard's Agar (RA) and Carrot Agar (CA) media were evaluated on the mycelial growth of the fungus (Sultana *et al.*, 2020). To find out an optimum temperature for the mycelia growth of the fungus, inoculated plates were incubated at 10°C, 15°C, 20°C, 25°C and 30°C for 7 days (Ahmmed *et al.*, 2020). The fungal culture media were adjusted to pH 5, 6, 7, and 8 with the addition of 1N NaOH or HCl before autoclave, and inoculated petri-plates were incubated at 25°C for 7 days (Sikder *et al.*, 2020).

Efficacy of bio-control agents, plant extracts, and fungicides against Curvularia lunata

The two rhizobacterial isolates *Bacillus subtilis* and *Pseudomonas fluorescens* and three fungal biocontrol agents- *Trichoderma harzianum*, *Trichoderma reesei* isolate 1 and *Trichoderma reesei* isolate 2 were assessed against the isolated fungal pathogen using duel culture technique (Bhadra *et al.*, 2016).

The ethanolic plant extracts were prepared from leaves of *Tagetes erecta* L., *Azardirachta indica* L., and *Lowsonia innermis* L. and each plant extract of different concentrations (10%, 20% and 30%) were mixed with PDA as different treatment combinations (Rahman *et al.*, 2015). Inoculated petri-plates were kept in an incubation chamber at 25±2°C. Radial growth of fungal mycelium was measured at 7 days post-incubation (dpi).

The effect of fungicides, namely Jazz 80 WP (Active ingredient: Ethylene bisdithiocarbamate), Amister Top 325 SC (active ingredients: Azoxystrobin + Difenoconazole), and Tilt 250 EC (active ingredients: Propiconazole) were assessed against *C. lunata*. A requisite quantity of fungicides was added to the medium to get concentration of 250 ppm, 500 ppm, and 750 ppm; inoculated plates were incubated at 25±2°C (Shamoli *et al.*, 2016). The fungal colony diameter was recorded at 7 dpi.

Percentage inhibition of the fungus was calculated by using the following formula: $I = ((C-T)/C) \times 100$. Here, I = percentage of mycelium growth inhibition; C = growth of mycelium in control; T = growth of mycelium in treatment.

Statistical analysis

Data generated in the experiment was checked for normality and homogeneity of variance; and analyzed using the SPSS program (SPSS Inc., Chicago, IL, USA). All parameters for inter-group differences were analyzed by Oneway ANOVA followed by post-hoc test.

Results and Discussion

Morphological and molecular identification of the fungus

Fungal colonies were fast-growing, brown to blackish brown with dark reverse. Conidiophores were erect, septate, unbranched, flexuose in the apical part, with flat, dark brown scars (Figure 1).

Conidia were septate, smooth-walled, olivaceous brown, end cells somewhat paler, obovoidal to broadly clavate shaped, curved at the subterminal cell (Figure 1). Based on morphological features, the studied organism was identified as *Curvularia* sp.

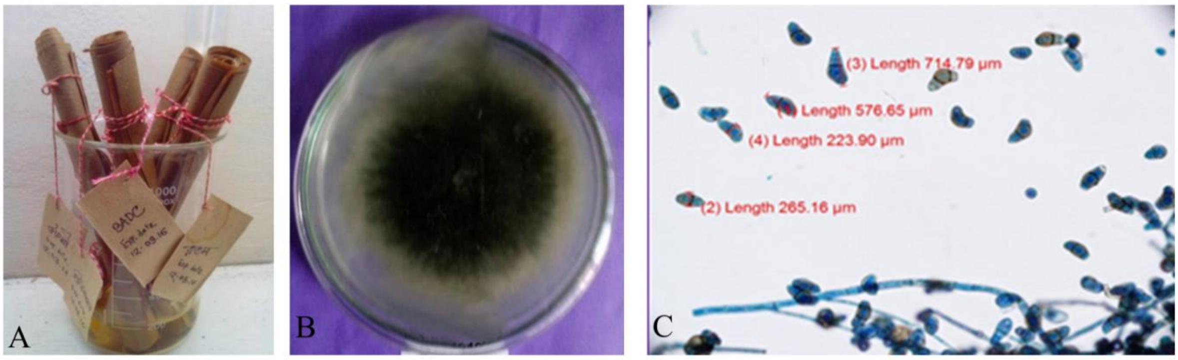

Figure 1. Incubation of tomato seeds in the paper towel (A), fungal colony on PDA medium (B), and microscopic view (400 X) of *C. lunata* (C).

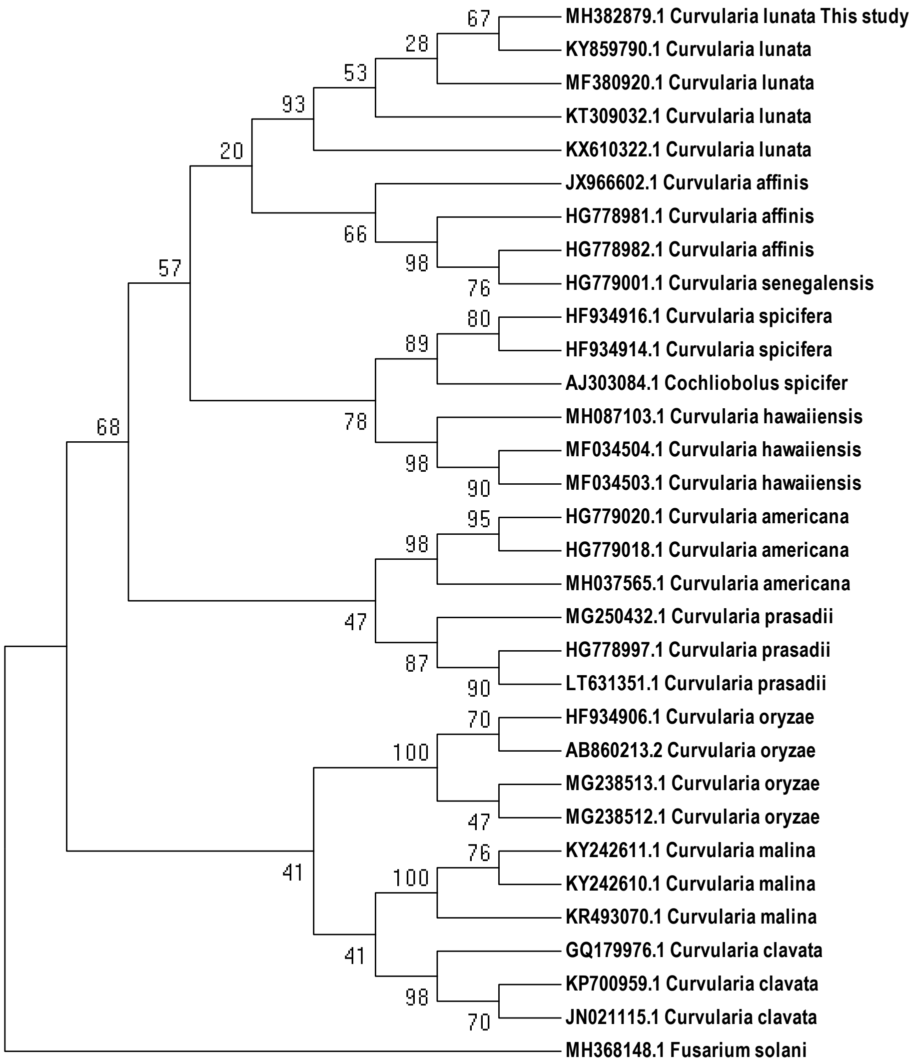

Figure 2. A neighbor-joining phylogenetic tree of the studied fungus along with other species of *Curvularia* with bootstrap value. Our fungus has been marked as this study.

To identify the fungus at species rank, sequencing of the ITS region was performed. In blast search, our fungus (MH382879.1: *Curvularia lunata*) showed above 99% sequence similarity with previously identified fungus- KX610322.1: *C. lunata,* MF380920.1: *C. lunata*, KT309032.1: *C. lunata*, and KY859790.1: *C. lunata*. The phylogenetic analysis was conducted via the multiple sequence alignment tools using MEGA 6 software (Figure 2). Phylogenetic tree confirmed the studied organism as a *C. lunata*, which formed a separate cluster with other *C. lunata*.

Effect of culture media on the mycelial growth of C. lunata

To find out the most suitable solid culture media for the growth and development of the fungus, the pathogen was grown on nine different solid media (Figure 3). Most of the culture media contains dextrose as a carbon source and peptone as a nitrogen source for mycelial growth and development. Our results revealed that Carrot agar, Kauffman's Agar, Honey peptone agar, and Potato sucrose agar were supported the maximum mycelial growth of *C. lunata* while yeast extract agar and potato dextrose agar media were less suitable to the fungus. Shabana *et al.* (2015) found the maximum colony diameter and growth rate of *Curvularia prasadii* on malt extract agar, followed by carrot agar and potato dextrose agar. Kumar *et al.* (2018) reported significantly higher mycelial growth of *C. lunata* on Sabouraud´s agar compared to PDA while other studies reported that *C. lunata* showed best vegetative growth on the PDA medium (Bhatt and Kumar, 2018). Similarly, Potato dextrose agar, Sabouraud's agar, and Host extract agar were best for the vegetative growth and sporulation of *C. lunata* (Sumangala and Patil, 2010). Our results suggest that HPA, KA, PSA, and CA are the most suitable fungal culture media of *C. lunata*.

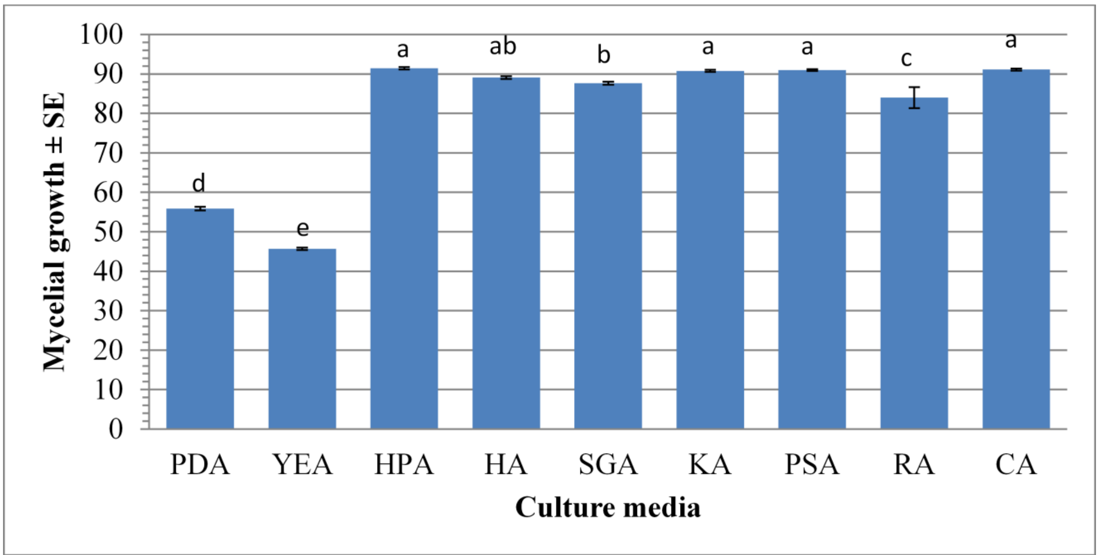

Figure 3. Effect of different fungal culture media on the mycelial growth of *C. lunata* at 7 dpi. PDA: Potato dextrose agar; YEA: Yeast Extract Agar; HPA: Honey Peptone Agar; MA: Malt Agar; HA: Hansen's Medium; SGA: Sabouraud's Glucose Agar; KA: Kauffman's Agar; PSA: Potato Sucrose Agar; RA: Richard's Agar; CA: Carrot Agar. The value represents as mean ± standard error (SE) of three replications. Means followed by a common letter (s) do not differ significantly at the 5% level by DMRT.

Effect of temperature on the mycelial growth of C. lunata

Among the environmental factors, temperature plays a vital role in the growth, development, and reproduction of fungi. Each fungus has its optimum temperature requirement. We aimed to know the effect of different incubation temperatures on the growth of *C. lunata* on PDA media (Figure 4). The highest mycelial growth of the fungus was recorded at 30^{0}C temperature, which was statistically different compare to other temperature conditions. With the decreasing temperature, the mycelial growth of *C. lunata* was also decreased and the lowest vegetative growth was recorded at 10^{0}C. Present findings are in agreement with the previous results. The most preferable temperature ranges was between 25 and 30^{0}C for vegetative growth of *C. lunata*, *Curvularia clavata, Curvularia pallescens*, *Curvularia trifolii* and *Curvularia aeria* (Almaguer *et al.*, 2013; Sumangala and Patil, 2010; Bhatt and Kumar, 2018; Lal *et al.*, 2014). Likewise, Shabana *et al.* (2015) also reported the best mycelial growth of CPO 1 and CPO 3 isolates of *Curvularia prasadii* was obtained at an incubation temperature of 30^{0}C.

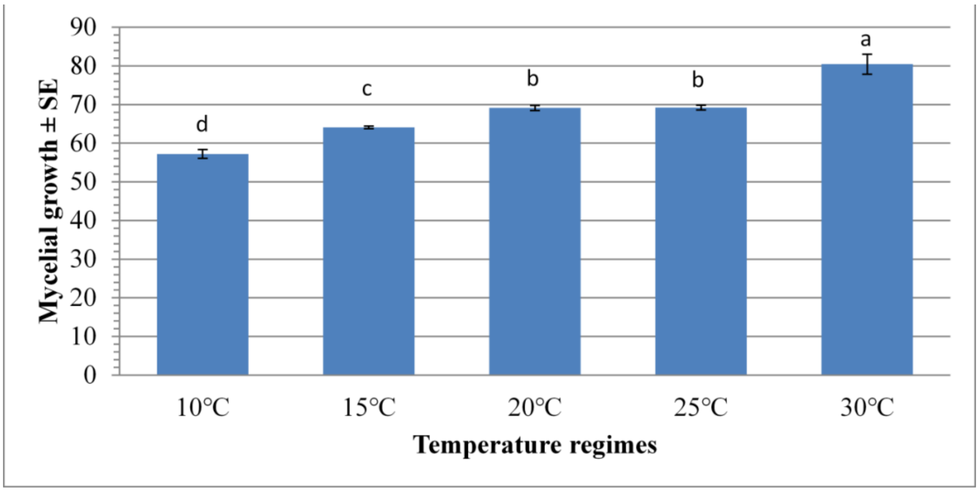

Figure 4. Effect of different temperatures on the mycelial growth *C. lunata* at 7 dpi. The value represents as mean ± standard error (SE) of three replications. Means followed by a common letter (s) do not differ significantly at the 5% level by DMRT.

Effect of pH on the mycelial growth of C. lunata

Different fungal pathogens require a particular pH for their growth and development. The growth rate of the different pathogens changed with the pH conditions of the fungal culture media. In the present study, an effort was made to investigate an optimum hydrogen ion concentration requirement for the *C. lunata*. Our results revealed that the maximum mycelial growth was obtained at pH 6, followed by pH 7 at 7 dpi (Figure 5). It was observed that *C. lunata* preferred more acid than alkali conditions. Presents study are supported by other researchers who reported the maximum growth of *C. lunata* (Sumangala and Patil, 2010) and *Curvularia pallescens* (Sonia *et al*., 1998) at pH 6. In another study showed that the pH levels between 6 and 8 induced the highest mycelial growth of *Curvularia pradasii* (Shabana *et al*., 2015).

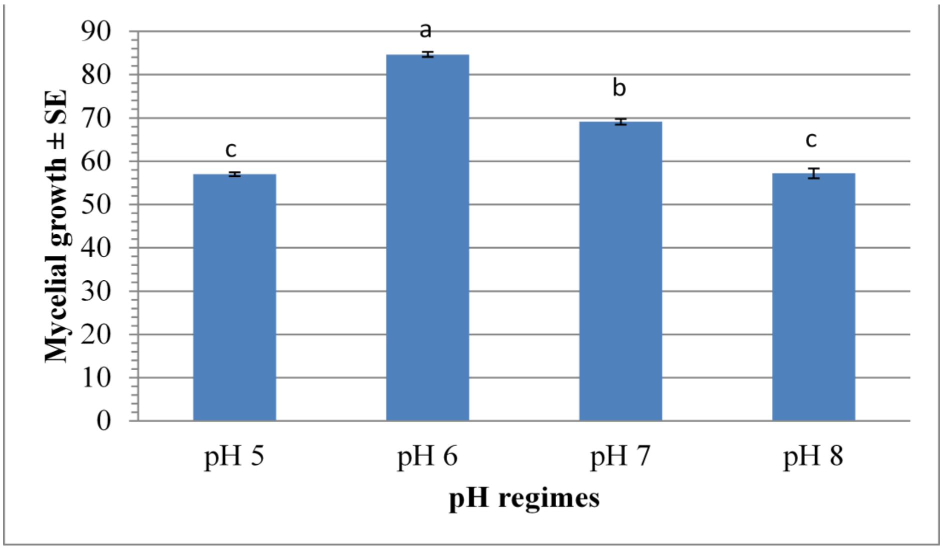

Figure 5. Effect of pH on the mycelium growth of *C. lunata* at 25±2°C temperature at 7 dpi. The value represents as mean ± standard error (SE) of three replications. Means followed by a common letter (s) do not differ significantly at the 5% level by DMRT.

Efficacy of plant extract on the mycelial growth inhibition of C. lunata

Results on the effect of plant extract on mycelial growth inhibition of *C. lunata* have been presented in Figure 6. The maximum mycelial growth inhibition was obtained due to the higher dose of *Lowsonia inermis*, followed by *Tagetes errecta* and *Azardirachta indica* at 7 dpi. Present findings are in conformity with the results of Barupal *et al.* (2019) who found that *L. inermis* inhibited the mycelial growth of 65% of *C. lunata* and chemical analysis revealed abundant quantities of volatile oils, flavonoids, steroids, and tannins contents in plant leaves. Similar mycelial inhibition of *C. lunata* was also observed by Mohana *et al.* (2011).

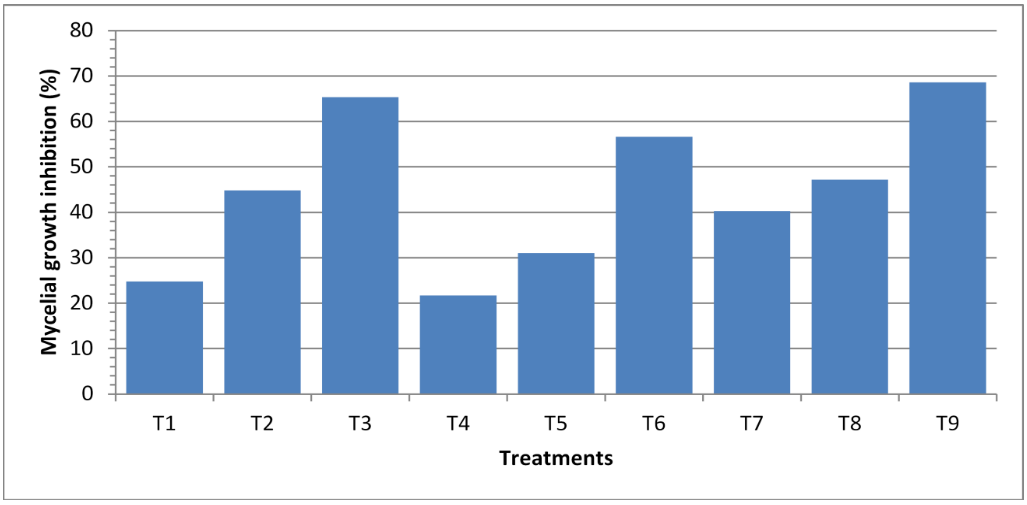

Figure 6. Effect of different ethanolic plant extracts on mycelial growth inhibition of *C. lunata* at 7 dpi. Here, T1: *T. erecta* (10%), T2: *T. erecta* (20%), T3: *T. erecta* (30%), T4: *A. indica* (10%), T5: *A. indica* (20%), T6: *A. indica* (30%), T7: *L. inermis* (10%), T7: *L. inermis* (20%), T7: *L. inermis* (30%).

Efficacy of bio-control agents on the mycelial growth inhibition of C. lunata

In our study, *C. lunata* was shown sensitivity to the antagonistic bio-control agents (Figure 7 and 8)**.** The maximum inhibition of mycelium growth of *C. lunata* was exhibited by *Bacillus subtilis* whereas *Pseudomonas fluorescens* was ineffective against the fungus. Importantly, *Trichoderma* sp. showed good results against *C. lunata*, in which 63.18%, 69.57%, and 54.89% mycelial inhibition was recorded due to *Trichoderma reesei* isolate 1, *Trichoderma reesei* isolate 2 and *Trichoderma harzianum*, respectively (Figure 8). Our results are supported by a study, in which sorghum rhizosphere originated *Bacillus* sp. showed higher antagonistic activity against *C. lunata*. Besides, scanning electron microscopic observations revealed hyphal lysis and degradation of the fungal cell walls (Basha and Ulaganathan, 2002). Tapwal *et al.* (2011) cited that *T. viride* showed the utmost mycelial inhibition of fungal pathogens including *C. lunata*. Anwar *et al.*, (2008) reported that *Trichoderma* spp. inhibit pathogenic invasion through phenomena of mycoparasitism, antibiosis and competition and lysis of pathogenic hyphae.

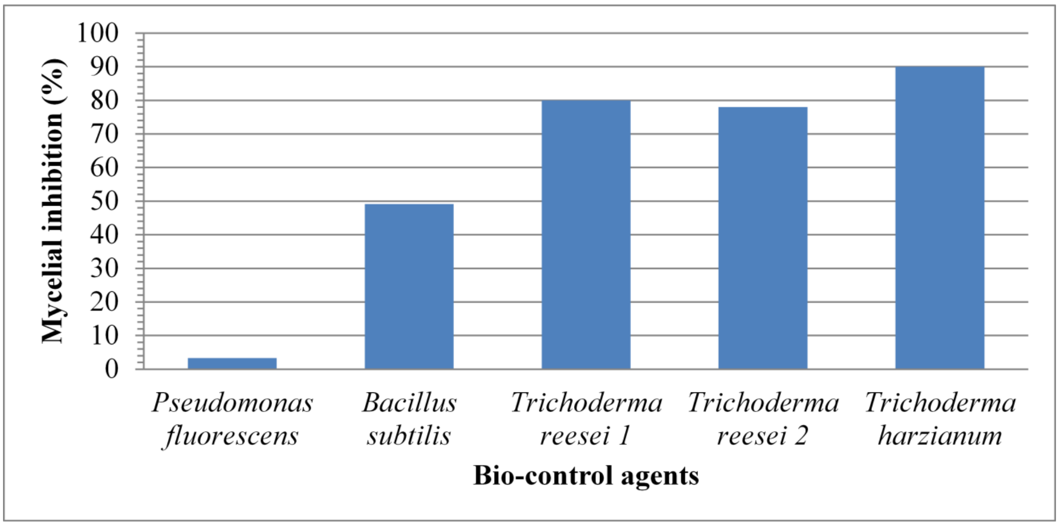

Figure 7. Effect of different antagonists on the mycelium growth inhibition of C. *lunata* at 25±2°C temperature.

Efficacy of fungicides on the mycelial growth inhibition of C. lunata

The different concentrations of three commercial fungicides (Jazz 80 WP, Amister Top 325 SC, and Tilt 250 EC) were evaluated on the mycelium growth inhibition of *C. lunata* under *in vitro* conditions. Among the three concentrations (250 ppm, 500 ppm, and 750 ppm) of fungicides, Tilt 250 EC showed the maximum inhibition of mycelium growth resulting in 100% inhibition due to all three concentrations at 7 dpi (Figure 8 and 9). Besides, Amister Top 325 SC also showed better performance compared to Jazz 80 WP against *C. lunata* with the inhibition 48.54%, 57.50%, and 64.41% due to 250 ppm, 500 ppm, and 750 ppm, respectively (Figure 8 and 9). Our results are agreement with the previous findings of Mamun *et al.* (2016) who also reported that Tilt 250 EC (Propiconazole) completely inhibited the radial growth of *C. lunata* at all concentrations (100, 200, 300, 400 and 500 ppm). Likewise, Chowdhury *et al.* (2015) evaluated Tall 25 EC (Propiconazole) fungicides at 100, 200, 300, 400 and 500 ppm against several pathogenic fungi *viz.* *Alternaria alternata*, *Curvularia lunata*, *Drechslera oryzae,* and *Pestalotiopsis guepinii* and found very promising against tested fungi at all the concentrations.

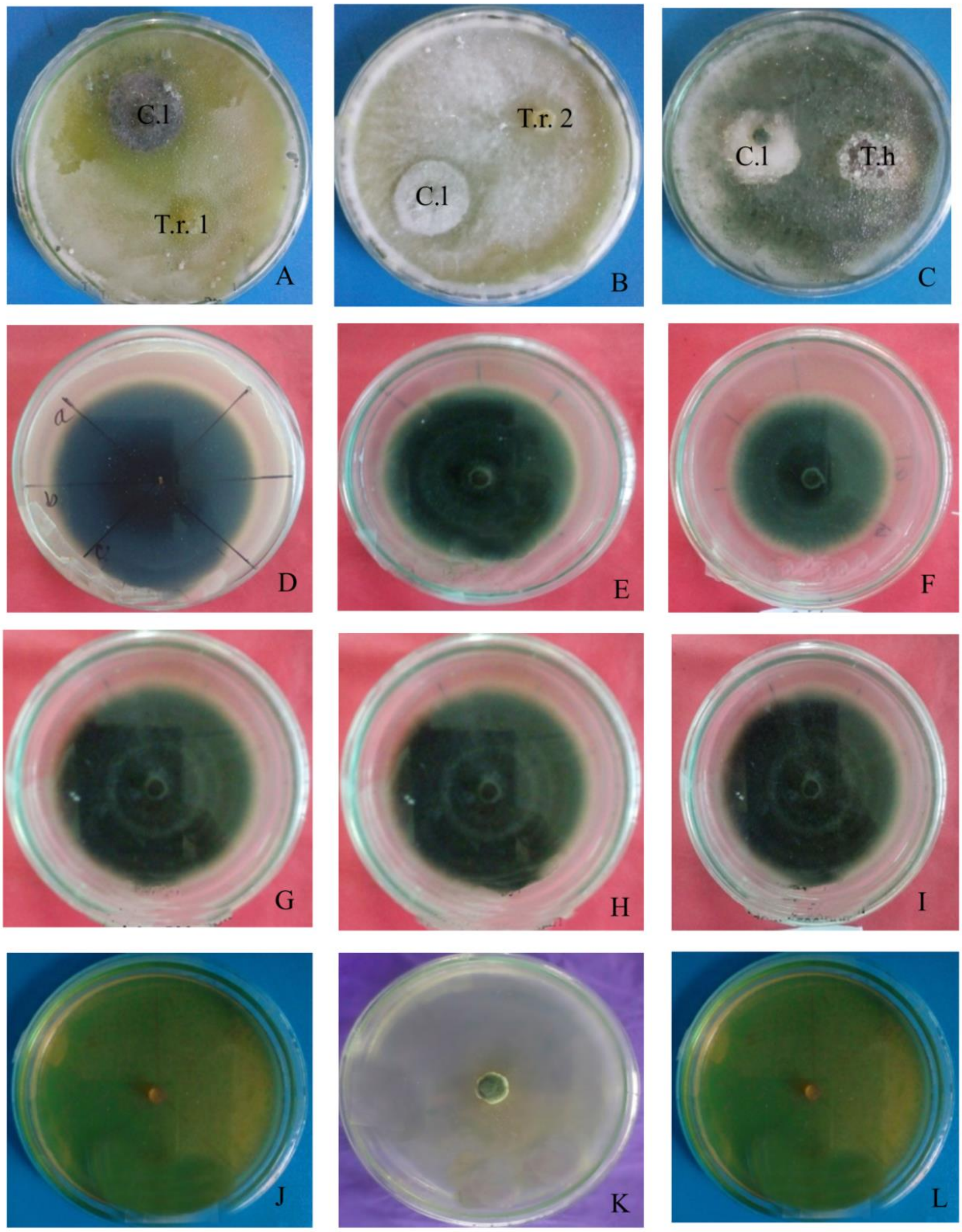

Figure 8. Photograph showing the effect of bio-control agents and fungicides on mycelial growth inhibition of *C. lunata* at 25±2°C temperature. Here, A: *Trichoderma reesei* isolate 1 (T.r 1) vs *C. lunata* (C.l); B: *Trichoderma reesei* isolate 2 (T. r 2) vs *C. lunata* (C.l); C: *Trichoderma harzianum* (T. h) vs *C. lunata* (C.l); D: Jazz 80 WP (250 ppm); E: Jazz 80 WP (500 ppm), F: Jazz 80 WP (750 ppm), G: Amister Top 325 SC (250 ppm), H: Amister Top 325 SC (500 ppm), I: Amister Top 325 SC (750 ppm), J: Tilt 250 EC (250 ppm), K: Tilt 250 EC (500 ppm), L: Tilt 250 EC (750 ppm).

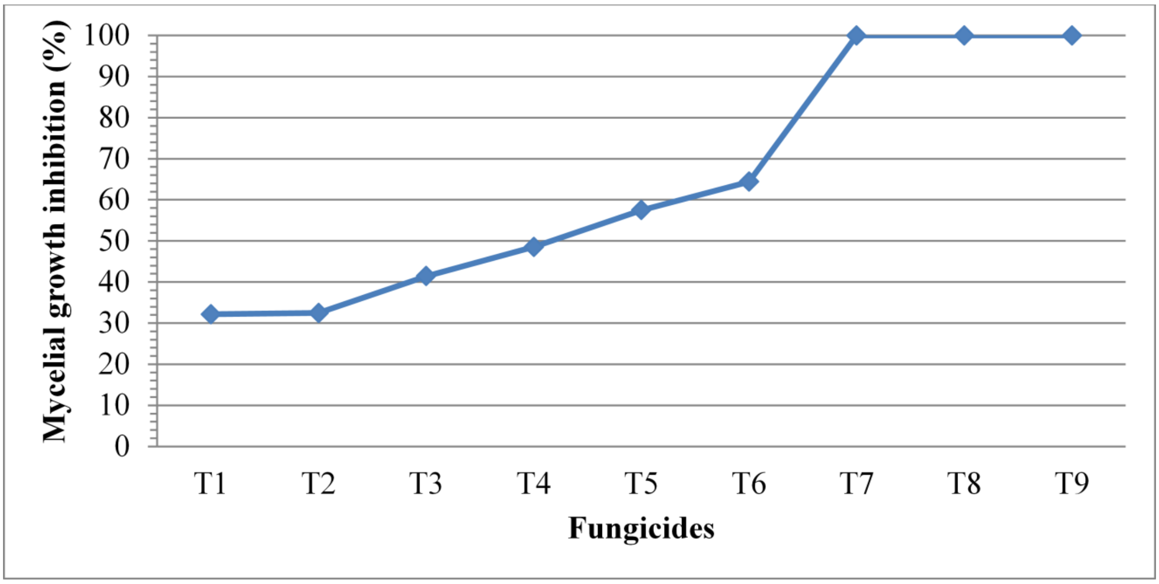

Figure 9. Effect of fungicides on mycelial growth inhibition (%) of *C. lunata* at 25±2°C temperature. Here, T1: Jazz 80 WP (250 ppm), T2: Jazz 80 WP (500 ppm), T3: Jazz 80 WP (750 ppm), T4: Amister Top 325 SC (250 ppm), T5: Amister Top 325 SC (500 ppm), T6: Amister Top 325 SC (750 ppm), T7: Tilt 250 EC (250 ppm), T8: Tilt 250 EC (500 ppm), T9: Tilt 250 EC (750 ppm).

Hossen *et al.* (2017) reported that Tilt 250 EC was known to reduce the incidence of fungal pathogens namely- *Aspergillus* spp., *Colletortichum capsici*, *Curvularia lunata*, and *Fusarium* spp., on chilli seeds. Besides, Tekade *et al.* (2017) cited that among all the systemic and non-systemic, and combination products, complete inhibition of *C. lunata* causing blight of coleus was achieved in propiconazole, carbendazim + mancozeb, tricyclozole + mancozeb, and zineb + hexaconazole.

References

Agarwal, V.K. 1981. Seed-borne fungi and viruses of some important crops. Research Bulletin, Govind Ballabh Pant Univ. Agric. Tech. 108: 147.

Ahmmed, S.M., 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. *Int. J. Bot. Stud.* 5(5): 164-169.

Almaguer, M., Rojas, T.I., Dobal, V., Batista, A. and Aira, M.J. 2013. Effect of temperature on growth and germination of conidia in *Curvularia* and *Bipolaris* species isolated from the air. *Aerobiologia*. 29: 13-20. https://doi.org/10.1007/s10453-012-9257-z

Anwar, A., Bhat, G.N. and Bhat, K.A. 2008. Mycoparasitic behaviour of certain bioagents against sheath blight pathogen (*Rhizoctonia solani*) of rice. *J. Mycol. Plant Path.* 38(1): 135–137.

Bagga, P.S. and Sharma, V.K. 2006. Evaluation of fungicides as seedling treatment for controlling bakanae/foot rot (*Fusarium moniliforme*) disease in Basmati rice. *Indian Phytopathol.* 59: 305–308.

Barupal, T., Meena, M. and Sharma, K. 2019. Inhibitory effects of leaf extract of *Lowsonia inermis* on *Curvularia lunata* and characterization of novel inhibitory compounds by GC-MS analysis. *Biotechnol. Rep.* 23: e00335. https://doi.org/10.1016/j.btre.2019.e00335

Basha, S. and Ulaganathan, K. 2002. Antagonism of *Bacillus* species (strain BC121) towards *Curvularia lunata*. *Curr. Sci.* 82(12): 1457-1463.

Bhadra, M., Khair, A., Hossain, M.A., Shamoli, F.A. and Sikder, M.M. 2016. Biological control of wilt of eggplant caused by *Fusarium solani* f.sp. *melongenae*. *Int. J. Exp. Agric.* 6(2): 20-25.

Bhatt, D. and Kumar, P. 2018. Effect of media, temperature, and light wavelength on the growth of *Curvularia lunata* causing *Curvularia* leaf spot of maize. *Int. J. Curr. Microbiol. Appl. Sci.* 7(9): 2227-2230. https://doi.org/10.20546/ijcmas.2018.709.275

Chowdhury, P.M., Bashar, M.A. and Shamim, S. 2015. *In vitro* evaluation of fungicides and plant extracts against pathogenic fungi of two rice varieties. *Bangladesh J. Bot.* 24(2): 251-259. https://doi.org/10.3329/bjb.v44i2.38514

Ellis, M.B. 1971. Dematiaceous Hyphomycetes (1st ed.). Commonwealth Mycological Institute, Kew, Surrey, UK. p. 608.

Gupta, S., Dubey, A. and Singh, T. 2017. *Curvularia lunata* as, a dominant seed-borne pathogen in *Dalbergia sissoo* Roxb: Its location in seed and its phytopathological effects. *African J. Plant Sci.* 11(6): 203-208. https://doi.org/10.5897/AJPS2017.1529

Hamim, I., Mohanto, D.C., Sarker, M.A. and Ali, M.A. 2014. Effect of seed borne pathogens on germination of some vegetable seeds. *J. Phytopathol. Pest Manage.* 1(1): 34-51.

Hossen, M.T., Sohag, M.A.S. and Monjil, M.S. 2017. Comparative efficacy of garlic, BAU-biofungicide, Bavistin and Tilt on seed borne fungal flora in chilli. *J. Bangladesh Agril. Univ.* 15(1): 41–46. https://doi.org/10.3329/jbau.v15i1.33528

Kumar, S.P., Mishra, M.K. and Sahu, K.C. 2018. Evaluation of culture media for growth characteristics of fungal leaf blight complex of tomato. *Int. J. Curr. Microbiol. App. Sci.* 7(11): 1073-1077. https://doi.org/10.20546/ijcmas.2018.711.124

Lal, M., Ali, M., Kumar, S., Singh, V. and Khan, A. 2014. Effect of media, nitrogen sources and temperature on the growth and sporulation of *Curvularia lunata* causing *Curvularia* leaf spot of Blackgram. *Bioscan.* 9(3): 1197-1199.

Mamun, M.A. Shamsi, S. and Bashar, M.A. 2016. *In vitro* evaluation of fungicides and plant extracts against pathogenic fungi on jute. *Biors. Comm.* 2(1): 189-192.

Mohana, D.C., Prasad, P., Vijaykumar, V. and Raveesha, K.A. 2011. Plant extract effect on seed-borne pathogenic fungi from seeds of paddy grown in Southern India. *J. Plant Prot. Res.* 51(2): 101-106. https://doi.org/10.2478/v10045-011-0018-8

Rahman, S.M.M., Sikder M.M., Nusrat, S. and Khair, A. 2015. *In vitro* evaluation of botanical extract, bioagents and fungicides against purple blotch diseases of bunch onion in Bangladesh. *Adv. Zool. Bot.* 3(4): 179-183. https://doi.org/10.13189/azb.2015.030403

Shabana, Y.M., Abou Tabl, A.H. and Sadek, M.E. 2015. Effect of nutrition and physical factors on mycelial growth and spore production of *Curvularia lunata,* a mycoherbicide agent barhyeard grass (*Echianochola crassgalli)* in rice. *J. Plant Prot. Path. Mansowra Univ.* 6(8): 1143-1153. https://doi.org/10.21608/jppp.2015.74715

Shamoli, F.A., Khair, A., Bhadra, M., Hossain, M.A. and Sikder, M.M. 2016. Symptomatology of fungal competitors on oyster mushroom's spawn packets and in vitro evaluation using phytoextracts and a fungicide. *Int. J. Agril. Res. Innov. Tech.* 6(2): 24-30. https://doi.org/10.3329/ijarit.v6i2.31701

Sikder, M.M., Ahmmed, S.M., Sultana, A. and Alam, N. 2020. First report on black spot disease of *Phyllanthus emblica* L. fruits caused by *Thielaviopsis paradoxa* in Bangladesh. *Int. J. Agril. Res. Innov. Tech.* 10(2): 38-46. https://doi.org/10.3329/ijarit.v10i2.51575

Sikder, M.M., Mallik, M.R.I. and Alam, N. 2019. Identification and *in vitro* growth characteristics of Entomopathogenic fungus-*Aschersonia* sp. in Bangladesh. *Adv. Zool. Bot.* 7(1): 11-18. https://doi.org/10.13189/azb.2019.070102

Sonia, V.P.F., Laura, M.P., Elza, A.L.A. and Leonor, C.M. 1998. Morphological, cytological, and cultural aspect of *Curvularia pallescens*. *Rev. Microbiol.* 29(3): 1-9. https://doi.org/10.1590/S0001-37141998000300010

Sultana, S., Sikder, M.M., Ahmmed, S.M., Sultana, A. and Alam, N. 2020. Mycelial growth and biological control measures of *Botrytis cinerea* isolated from Strawberry fruit rot disease in Bangladesh. *Univ. J. Microbiol. Res.* 8(2): 13-18. https://doi.org/10.13189/ujmr.2020.080201

Sumangala, K. and Patil, M.B. 2010. Cultural and physiological studies on *Curvularia lunata*, a casual agent of grain discolouration in rice. *Int. J. Plant Prot.* 3(2): 238-241.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol. Biol. Evol.* 30(12): 2725-2729. https://doi.org/10.1093/molbev/mst197

Tapwal, A., Singh, U., Jaime, A., da Silva, T. Singh, G., Garg, S. and Kumar, R. 2011. *In vitro* antagonism of *T. viridae* against five phytopathogens. *Pest. Tech.* 5(1): 59-62.

Tekade, A., Koche, M.D., Kothikar, R.B. and Surpam, A.N. 2017. Efficacy of fungicides and bioagents against *Curvularia lunata* causing blight of coleus under laboratory conditions. *J. Med Plant Stud.* 5(2): 189-19.

Tyagi, S. and Paudel, R. 2014. Effect of different pH on the growth and sporulation of *Fusarium oxysporum*: The causal organism of wilt disease of Tomato. *Int. J. Basic Appl. Biol.* 2(1): 103-106.

Vijendra, K.A. and Sinclair, J.B. 1997. Principles of Seed Pathology (2nd Edn.), CRC Press, Boca Raton. p. 92.

White, T.J., Bruns, T., Lee, S. and Taylor, J.W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. pp. 315-322 In: PCR Protocols: A Guide to Methods and Applications, eds. Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. Academic Press, Inc., New York.

Yadav, R.S., Tyagi S., Javeria S. and Gangwar R.K. 2014. Effect of different cultural condition on the growth of *Fusarium moniliforme* causing Bakanae disease. *Eur. J. Mol. Biotech.* 4(2): 95-100. https://doi.org/10.13187/ejmb.2014.4.95