Int.J.Curr.Microbiol.App.Sci (2018) *7*(4): 627-635

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 04 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.704.070

Mycelia Growth and Spore Yield of *Trichoderma harzianum* in Batch and Fed-Batch Cultures: Influence of pH and Temperature

Abiodun A. Onilude and Damilola O. Seyi-Amole*

Microbial Physiology and Biochemistry Laboratory, Department of Microbiology, University of Ibadan, Ibadan, Nigeria

**Corresponding author*

Keywords

Trichoderma harzianum, Batch culture, Fed-batch culture, pH, Temperature

Article Info

Accepted:
07 March 2018
Available Online:
10 April 2018

A B S T R A C T

Trichoderma harzianum is a biocontrol agent that has moderate effect on soil balance and no harmful effect on other beneficial organisms in the soil. In this study we examined the effect of pH and temperature on the mycelia growth and spore yield of *T. harzianum* in batch and fed-batch culture. The pH and temperature had significant effect on the growth and sporulation of *T. harzianum* and this was dependent on the cultivation technique. It was observed that the optimum pH for maximum mycelial growth and spore yield produced by *T. harzianum* in batch and fed-batch cultures was pH 4 while the optimum temperature for mycelial growth in batch and fed-batch culture was 30 ^{o}C and the maximum spore yield was produced at optimum temperature of 25 ^{o}C and 45 ^{o}C in batch and fed- batch culture respectively. The results showed that fed-culture produced more spore yield than batch culture while high mycelial growth was obtained in batch culture. This work has revealed the important role that environmental conditions plays in the mycelia growth and spore yield of *T. harzianum*, a biocontrol agent.

Introduction

Trichoderma species are the most common saprophytic fungi in the rhizosphere and are found in almost any soil. They have mycoparasitic ability against economically important aerial and soil borne plant pathogens (Dubey *et al.*, 2007; Kuhls *et al.*, 1996; Papavizas and Lumsden, 1980). However, *Trichoderma* spp. have been of great interest to many researchers who have been contributing to biological control pursuit through the use of fungi (Ortiz and Orduz, 2001; Heraux *et al.*, 2005). *Trichoderma* strains have been successfully used as biological control agents (BCAs) due to their high adaptability and reproducibility in diverse conditions, efficiency in the utilization of nutrients, ability to modify the rhizosphere, antagonistic activity against phytopathogenic fungi which promotes plant growth and defense mechanisms (Chet *et al.*, 1997). *T. harzianum* is a common BCA, used as against phytopathogenic and viral vector fungi (Grondona *et al.*, 1997).

Generally, fungal spores (especially, conidia), are preferably used for commercial production of fungal BCAs because they are more tolerant to adverse environmental conditions

during product formulation and field use, in contrast to their mycelia and chlamysdospore forms as microbial propagules (Amsellem *et al.,* 1999). However, the presence of mycelia along with conidia would insure presence of various essential metabolites (e.g., antibiotics) for BCA activity (Roberts *et al.,* 2005).

When planning the application of biocontrol strains, it is very important to consider the environmental stresses affecting microbial activities. As in all microorganisms even in *Trichoderma*, the external factors modify their morphological characteristics as well as physiological functions. Among these factors, pH and temperature are probably the most important environmental parameter affecting the mycoparasitic activities of *Trichoderma* strains (Kredics *et al.,* 2004). Biocontrol strains should have better stress tolerance levels than the plant pathogens against which they are going to be used during biological control. Therefore, it is also of great importance to collect information about the effects of pH and temperature on mycelia growth and spore yield of *T. harzianum* in order to know conditions that will favour high production yield *T. harzianum* propagules for BCAs.

Liquid fermentation has been pursued by researchers than solid state fermentation due to its compatibility with pre-existing large scale facilities. Of the different types of liquid fermentation, fed-batch systems appear to have potential for commercial spore production by fungi (Casino *et al.,* 1990; Molla *et al.,* 2004). Unfortunately accurate information regarding the factors affecting the production of spores in liquid culture still remains a challenging task and warrants considerable research inputs. This study was designed to examine the influence of pH and temperature on mycelial growth and sporulation of *T. harzianum* in batch and fed-batch cultures.

Materials and Methods

Fungal culture

The fungal culture of *T. harzianum* was obtained from The Culture Collection Centre of The Department of Microbiology University of Ibadan, Nigeria. The strain was maintained on Potato dextrose agar (PDA).

Batch and fed-batch culture fermentation

Mycelium growth and spore yield of *T. harzianum* was studied in batch and fed-batch cultures. 50mL of the liquid medium described by Al-Taweil *et al.,* (2009), which contained (g/L) ammonium chloride (2.0), sodium potassium tartrate (2.0), $MgSO_4.7H_2O$ (4.0), K_2HPO_4 (14.0), $CaCl_2$ (0.2), KH_2PO_4 (4.0), yeast extract (4.5), trace element (2.0 ml), [$ZnSO_4.7H_2O$ (0.0014), $FeSO_4.7H_2O$ (0.005), $MnSO_4$ (0.0016), $CoCl_2$ (0.002)], glucose (7.5), $NaNO_3$ (6.0), and corn steep liquor (5.0) was used for both batch and fed-batch culture fermentation.

Batch culture fermentation was done as described by Cascino *et al.,* (1990). 250 mL Erlenmeyer flask containing 50 mL of liquid medium was inoculated with 1 mL of the spore suspension prepared according to the method of Nahar *et al.,* (2008) and was later incubated at specified temperature under static condition in the dark for 7 days.

Fed-batch culture fermentation was conducted using the modified method of Cascino *et al.,* (1990). A fed-batch vessel containing 50 mL of liquid medium was inoculated with 1 mL of the spore suspension and incubation at specified temperature under static condition for 7 days.

At every 12 h intervals, 4 mL of the limiting nutrient (yeast extract 0.05 mg/mL) was added.

Influence of pH

For the influence of various pH levels on mycelial growth and sporulation, the growth medium was prepared using citrate buffer regulated to varying pH, ranging from 3.0 to 6.0. 1 mL spore suspension of *T. harzianum* was inoculated into Erlenmeyer flask (250 mL) containing 50 mL of liquid medium and incubated at 30^0C for both batch and fed-batch cultures. The setup was replicated thrice after which the mycelial dry weight and spore yield was recorded after 7 days of incubation (Steyaert *et al.,* 2010a).

Influence of temperature

The influence of various temperatures on mycelial growth and sporulation was done according to the modified method of Schemaeza, *et al.,* (2013). Fifty milliliters of the liquid medium adjusted to pH 5.5 using citrate buffer was dispensed into 250 mL Erlenmeyer flasks. The liquid medium was later inoculated with 1mL spore suspension of *T. harzianum* and incubated at four different temperatures, viz, 25, 30, 37 and 45 ^{0}C for 7 days in both batch culture and fed-batch cultures. The setup was replicated thrice. Mycelial dry weight and spore yield was determined.

Spore yield determination

The spore yield was determined using the modified method of Waghunde *et al.,* (2010). Each flask was harvested by filtering the spore suspension through a sterilized double layered muslin cloth. The stock suspension was kept on Rotary Flask Shaker (MAC, MSW-301) for 2 minutes, after which 3mL of the suspension was added into a cuvette. The equipment was calibrated with 3 mL of blank solution (liquid medium). The spore yield was determined at a wavelength of 550 nm using Perkin Elmer Lambda 25 UV Spectrophotometer.

Assessment of dry cell mass

The fungus biomass yield was assessed by collecting fungal biomass on pre-weighed filter paper after incubation. The mycelium dry weight was determined after drying at 80 °C to constant mass. The actual weight of fungal mycelium was calculated by difference (Al-Taweil *et al.,* 2009).

Results and Discussion

Influence of pH

The effect of pH on the mycelia growth and spore yield by *T. harzianum* after 7 days in batch and fed-batch culture are shown in Figure 1 and 2. Analysis revealed that pH 4.0 supported the maximum mycelial growth of 14.29±0.56 g/L in batch culture while the lowest mycelial growth of 9.97±0.38 g/L was recorded at pH 6.0 (Figure 1a). Mycelial growth of *T. harzianum* was favourable within the optimum pH range of 3.5-5.0 in batch culture. Similarly, the maximum spore yield with optical density (OD) of 1.05±0.02 was supported at pH 4.0 while the lowest spore yield with OD of 0.17±0.03 was produced at pH 3.0 (Figure 1b).

Results of effect of initial pH in fed-batch culture are shown in Figure 2 reveals that pH 4.0 supported the highest mycelial growth of 11.92±0.43 g/L while the lowest mycelial growth of 3.28±0.61 g/L was recorded at pH 6.0 (Figure 2a).

The result reveals that optimum pH range of 3.5-5.0 favours the mycelial growth of *T. harzianum* in fed-batch culture. Similarly, the highest spore yield with OD of 1.24±0.05 was recorded at pH 4.0 while the lowest spore yield with OD of 0.21±0.12 was recorded at pH 3.0 (Figure 2b). The optimum pH range that favoured high spore yield of *T. harzianum* in fed-batch culture is pH 4.0-5.5.

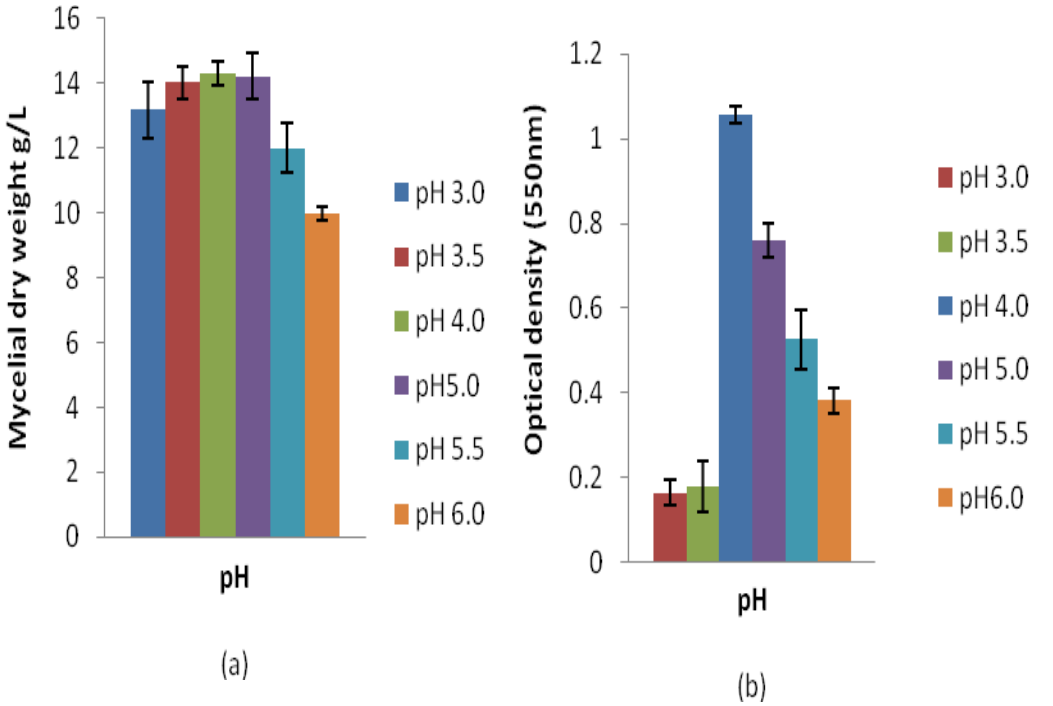

Fig.1 Influence of pH on the mycelial growth and spore yield of *T. harzianum* after 7days in batch culture. (a) Influence of pH on the mycelial growth of *T. harzianum*; (b) Influence of pH on the spore yield of *T. harzianum*. Data are means of three replicates. Mean± SEM

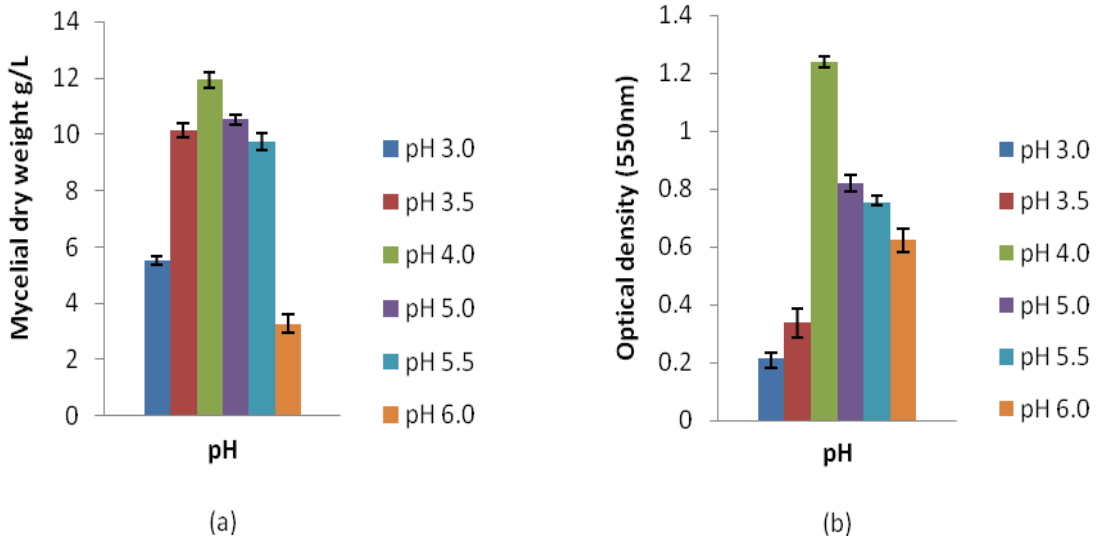

Fig.2 Influence of pH on the mycelial growth of *T. harzianum* after 7days in fed-batch culture. (a) Influence of pH on the mycelial growth of *T. harzianum*; (b) Influence of pH on the spore yield of *T. harzianum*. Data are means of three replicates. Mean± SEM

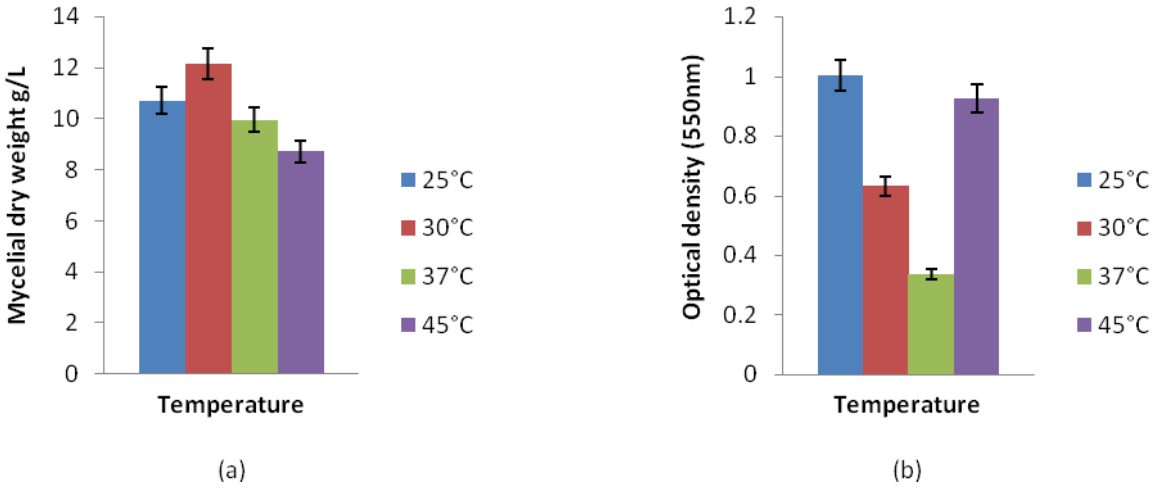

Fig.3 Influence of temperature on the mycelial growth and spore yield of *T. harzianum* after 7days in batch culture. (a) Influence of temperature on the mycelial growth of *T. harzianum*; (b) Influence of temperature on the spore yield of *T. harzianum*.
Data are means of three replicates. Mean± SEM

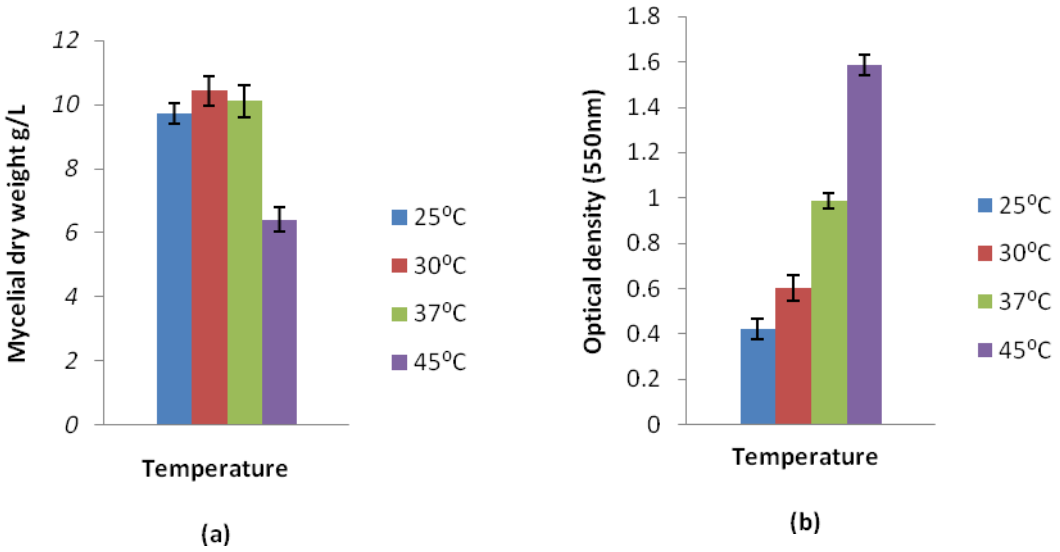

Fig.4 Influence of temperature on the mycelia growth and spore yield of *T. harzianum* after 7days in fed-batch culture. (a)Influence of temperature on the mycelium of *T. harzianum;* (b) Influence of temperature on the spore yield of *T. harzianum*.
Data are means of three replicates. Mean± SEM

Influence of temperature

The effect of temperature on the mycelial growth and spore yield by *T. harzianum* after 7 days in batch and fed-batch culture are shown in Figure 3 (A and B) and 4 (A and B). Analysis revealed that temperature 30 °C is the optimum temperature for mycelial growth

with a mycelial weight of 12.13±0.29 g/L in batch culture while the lowest mycelial growth of 8.72±0.56 g/L was recorded at 45 ^{o}C (Fig. 3A). Mycelial growth of *T. harzianum* was favourable within the optimum temperature range of 25-30 ^{o}C in batch culture. The highest spore yield with OD of 1.0±0.04 was supported at 25 ^{o}C while the lowest spore yield with OD of 0.34±0.11 was produced at 37 ^{o}C (Fig. 3B).

Results of effect of temperature in fed-batch culture are shown in Figure 4 (A and B) and it reveals that the optimum temperature that supported the highest mycelial growth of 10.43±0.38 g/L was 30 ^{o}C while the lowest mycelial growth of 6.40±0.25 g/L was obtained at 25 ^{o}C (Fig. 4A). The result reveals that optimum temperature of 25-37 ^{o}C favours the mycelial growth of *T. harzianum* in fed-batch culture. The spore yield with OD of 1.58±0.02 was highest spore yield produced and it was obtained at 45 ^{o}C while the lowest spore yield with OD of 0.42±0.03 was recorded at 25^{o}C (Fig. 4B). The optimum temperature range that favoured high spore yield of *T. harzianum* in fed-batch culture was 30-45 ^{o}C.

The growth and conidiation of *Trichoderma* is influenced by some known environmental factors include the ambient pH of the medium, temperature extracellular calcium, physical injury to the mycelium and the presence of fungal-derived volatile organic compounds (Steyaert *et al.,* 2010a). In our study, two fermentation techniques; batch and fed-batch culture, and different pH and temperature conditions affected the mycelial growth and conidiation of *T. harzianum*. The result of this study showed that in batch and fed-batch culture the optimum pH for mycelial growth and spore yield was pH4. The pH range that favoured the mycelial growth and spore yield was pH 3.5-5 and pH 4-5.5 respectively. It has been demonstrated that *Trichoderma* strains are active under a wider range of pH (Kredics *et al.,* 2003) and from our analysis it can de deduced that *T. harzianum* grow and sporulate maximally at a low ambient pH. This result is in line with the work of Papavizas *et al.,* (1982) and Steyaert *et al.,* (2010b). A similar response was observed in another study, it is found that *T. harzianum* grow in wide range of pH 2.0 to 6.0, with maximum growth at 4.0 (Kredics, 2004). The initial pH of the medium has also been demonstrated to have an effect on mycelial growth and conidiation, and unlike the C: N ratio, pH levels which favour conidiation have been shown to favour mycelia growth as well (Aube and Gagnon, 1969; Bastos, 2001; Brian and Hemming, 1950; Lewis and Papavizas, 1983; Steyaert *et al.,* 2010b). Benitez (2004) reported that growth of *Trichoderma* is more efficient in acidic than alkaline soils and they modify the rhizosphere soil by acidifying the soil. Low ambient pH of the growth medium has been demonstrated to result in intracellular acidification in *Aspergillus niger* and *Saccharomyces cerevisiae* Caspani *et al.,* 1985; Gradisnik-Grapulin and Legisa, 1997). This explains the reason why the *T. harzianum* strain prefered acidic pH.

Investigating the effect of temperature on *T. harzianum* in batch and fed batch culture showed that the optimum temperature for mycelial growth is 30^{o}C while the optimum temperature for spore yield in batch and fed-batch culture is 25^{o}C and 45^{o}C respectively. It can be inferred that *T. harzianum* has a broad range of tolerance as regards its growth and sporulation and that higher temperatures favours sporulation in fed-batch cultures. Maximum mycelial growth at the optimum temperatures in both batch and fed-batch cultures could be because it also affects their metabolic activity especially the production of volatile antibiotics and enzymes (Tronsmo and Dennis, 1978). Though temperature plays

an important role in the growth of organisms, at elevated level, it damages the organisms by denaturing enzymes, transport carriers, integrity of cell membrane (Prescott *et al.*, 2002).

In general, commercial preparations of *Trichoderma* spp. for biological control consist of bulk-produced conidia (asexual spores), whereas good biocontrol activity in the environment relies upon the fungus remaining vegetative, and thus antagonistically active. The result from this present study suggests that there are considerable and unexpected differences between the mycelia growth and spore yield by *T. harzianum* in batch and fed-batch cultures. However, fed-batch culture produced more spore yield than batch culture, this is in line with the work of Onilude *et al.*, (2013). Although more mycelial was produced in batch culture, unfortunately mycelia cannot survive downstream processing steps such as drying and hence are not useful as compared to conidia (Papavizas *et al.*, 1982). The high spore yield obtained in fed-batch culture was at the expense of greatly reduced mycelia, resulting in a high spore yield. The substrate-limited, fed-batch culture increased the amount of spore production by favouring the allocation of nutrients to spore production rather than mycelia production (Cascino *et al.*, 1990).

In conclusion, this work has shown that fed-batch system at acidic pH and temperature range of 30-45 ^{o}C will be the best production strategy for maximum spore production of *T. harzianum* for BCA application than batch culture system.

References

Al-Taweil, H. I., Osman, M. B., Aidil, A. H., and Yussof, W. M. W. 2009. Optimizing *Trichoderma viride* Cultivation in Submerged State Fermentation. Am. J. Appl. Sc. 6(7): 1277–1281.

Amsellem, Z., Zidack, N. K., Quimby, P. C., and Gressel, J. 1999. Long-term dry preservation of viable mycelia of two mycoherbicidal organisms. Crop Prot. 18: 643–649.

Aube, C., and Gagnon, C. 1969. Effect of carbon and nitrogennutrition on growth and sporulation of *Trichoderma viride* Pers ExFries. Can. J. Microbiol. 15: 703–706.

Bastos, C. N. 2001. Effect of temperature, pH and nutrition on growth and sporulation of *Trichoderma stromaticum* sp. nov., anantagonist of cocoa witches' broom pathogen. Summa Phytopathol. 27: 73–76.

Benitez, T. 2004. Increased antifungal and chitinase specific activates of *Trichoderma harzianum* CECT 2413 by addition of a cellulose binding-domain. Appl. Microbiol. Biotechnol. 64: 675-685.

Brian, P. W., and Hemming, H. G. 1950. Some nutritional conditions affecting spore production by *Trichoderma viride* Pers ex Fries. Trans. Br. Mycol. Soc. 33: 132–141.

Cascino, J. J., Harris, R. F., Smith, C. S., and Andrew, J. H. 1990.Spore yield and Microcycle Conidiation of *Colletotrichum gloeosporioides* in Liquid Culture. Appl. Environ. Microbiol. 56(8): 2303-2310.

Caspani, G., Tortora, P., Hanozet, G. M., and Guerritore, A. 1985. Glucose-stimulated cAMP increase may be mediated by intracellular acidification in *Saccharomyces cerevisiae*. FEBS Lett. 186: 75–79.

Chet, I., Inbar, J., and Hadar, I. 1997. Fungal antagonists and mycoparasites. In: Wicklow, D. T., and Soderstrom, B. (Eds.), The Mycota IV: Environmental

and microbial relationships, Springer-Verlag, Berlin, pp. 165-184.

Dubey, S.C., Suresh, M., and Singh, B. 2007. Evaluation of *Trichoderma* species against *Fusarium oxysporum* f. sp. ciceris for integrated management of chickpea wilt. Biol. Contr. 40:118-127.

Gradisnik-Grapulin, M., and Legisa, M. 1997. A spontaneous change in the intracellular cyclic AMP level in *Aspergillus niger* is influenced bythe sucrose concentration in the medium and by light. Appl. Environ. Microbiol. 63:2844–2849.

Grondona, I., Hermosa, M. R., Tejeda, M., Gomis, M. D., Mateos, P. F., Bridge, P.D., Monte, E., and Garciaacha, I. 1997. Physiological and biomedical characterisation of *Trichoderma harzianum*, a biological control agent against soil-borne fungal plant pathogens. Appl. Environ. Microbiol. 63: 3189-3198.

Heraux, F. M. G., Hollett, S. G., and Weller, S. C. 2005. Combining *Trichoderma virens*- inoculated compost and a rye cover crop for weed control in transplanted vegetables. Biol. Control. 34: 22-26.

Kredics, L., Antal, Z., Manczinger, L., and Szekeres, 2003. A. Influence of Environmental Parameters on *Trichoderma* Strains with Biocontrol Potential. Food Technol. Biotechnol. 41 (1): 37- 42.

Kredics, L., Manczinger, L., Antal, Z., Penzes, Z., Szekeres, K., Kevel, F., and Nagy, E. 2004. In vitro water activity and pH dependence of mycelial growth and extracellular enzyme activities of *Trichoderma* strains with biocontrol potential, J. Appl. Microbiol. 96: 491-498.

Kuhls, K., Lieckfeldt, E., Samuels, G. J., Kovacs, W., Meyer. W., Petrini, O., Gams, W., Borner, T., and Kubicek, C. P. 1996. Molecular evidence that the asexual industrial *fungus Trichoderma reesei* is a clonal derivative of the *ascomycete Hypocrea Jecorina.* Proc. Natl. Acad. Sci. U.S.A. 93: 7755-7760.

Lewis, J. A., and Papavizas, G. C. 1996. Production of chlamydospores and conidia by *Trichoderma* spp. in liquid and solid growth media. Soil Biol. Biochem. 15: 351–357.

Molla, A. H., Fakhru'l-Razia, A., Hanafi, M. M., and Alam, M. Z. 2004. Optimization of process factors for solid-state bioconversion of domestic wastewater sludge. Int. Biodeterior. Biodegrad. 53*:* 49-55.

Nahar, S., Hossain, F., Feroza, B., and Halim, M. A. 2008. Production of glucoamylase by *Rhizopus* sp. in liquid culture. Pak. J. Bot. 40(4): 1693–1698.

Onilude, A. A., Adebayo-Tayo, B. C., Odeniyi, A. O., Banjo, D., and Garuba, E. O. 2013. Comparative mycelial and spore yield by *Trichoderma viride* in batch and fed-batch cultures. Ann. Microbiol. DOI 10.1007/s13213-012-0502-z.

Ortiz, A., and Orduz, S. 2001. *In vitro* evaluation of *Trichoderma* and *Gliocladium* antagonism against the symbiotic fungus of the leaf-anting Alt Cephalotes. Mycopathologia. 150: 53-60.

Papavizas, G. C., and Lumsden, R. D. 1980. Biological control of soilborne fungal propagules. Annu Rev. Phytopathol. 18: 389-413.

Papavizas, G. C., Lewis, T. H., and Abd-El, M. 1982. Evaluation of new biotypes of T*richoderma harzianum* for tolerance to benomyl and enhanced biocontrol capabilities. Phytopathol. 72: 126-132.

Prescott, L. M., Harley, J. P., and Klein, D. A. 2002. Microbiology, fifth ed. The McGraw-Hill Companies Inc: North America. pp.1026.

Roberts, D. P., Lohrke, S. M., Meyer, S. L. F., Buyer, J. S., Bowers, J. H., Baker, C. J., Li, W., de Souz, J.T., and Lewis, J. A. 2005. Biocontrol agents applied individually and in combination for supprersion of soilborne disease of cucumber. Crop Prot. 24: 141-155.

Schemaeza, B., Somda, I., Sereme, P., Adam, T., and Ouedraogo, R. A. 2013. Effects of Temperature and pH on Mycelium Growth of *Phoma sorghina* (Sacc.) Boerema Dorenbosch and Van Kesteren *in vitro*. Pak. J. Biol. Sci. 16*:* 2054-2057.

Steyaert, J. M., Weld, R. J., and Stewart, A. 2010b. Ambient pH intrinsically influences *Trichoderma* conidiation and colony morphology. Fungal Biol. 114: 198-208.

Steyaert, J. M., Weld, R. J., Mendoza-Mendoza, A., and Stewart, A. 2010a. Reproduction without sex: conidiation in thefilamentous fungus *Trichoderma*. Microbiol. 156: 2887–2900.

Tronsmo, A., and Dennis, C. 1978. Effect of temperature on antagonistic properties of *Trichoderma* species. Trans. Br. Mycol. Soc. 71: 469–474.

Waghunde, R. R., Priya, J., Naik, B. M., Solanky, K. U., and Sabalpara, A. N. 2010. Optical density – a tool for estimation of spore count of *Trichoderma viride*. J. Biopest. 3(3): 624-626.

How to cite this article:

Abiodun A. Onilude and Damilola O. Seyi-Amole. 2018. Mycelia Growth and Spore Yield of *Trichoderma harzianum* in Batch and Fed-Batch Cultures: Influence of pH and Temperature. *Int.J.Curr.Microbiol.App.Sci.* 7(04): 627-635. doi: https://doi.org/10.20546/ijcmas.2018.704.070