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REVIEW ARTICLE

LARGE SCALE PRODUCTION AND INCREASED SHELF LIFE OF TRICHODERMA HARZIANUM INOCULUMS IN SEMI SOLID MEDIUM

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ABSTRACT

Trichoderma harzianum is a well-known bio control agent that is commercially produced to prevent development of several soil borne plant pathogen. In addition to control of plant diseases, T. harzianum also promotes plant growth. Solid and liquid state fermentation methods are commonly used for mass production of T. harzianum inoculums. Solid fermentation is expensive as it requires substrate for fermentation. Liquid fermentation is also undesirable due to increase of pH, chemical degradation and lower shelf life over the time of storage. Therefore, an alternative semi solid medium has been developed for large scale fermentation of T. harizanum inoculums. The newly developed medium showed more cell count of T. harizanum and less chemical changes (most probably oxidation) over time compared to solid or liquid media. Two to three days after fermentation, the newly developed semi solid medium showed less production of gas compared to other media. Therefore, the newly developed semi solid medium could be used to increase the quality and quantity of T. harizanum inoculums for large scale commercial production.

KEYWORDS

Trichoderma harzianum, semi solid medium, large scale production, biopesticide

1. INTRODUCTION

Trichoderma was first classified as fungi in 1794 and observed as problematic and embraced alternative of several fungi like *Puccinia, Mucor, Ascobolus* and a few slime molds. This concept leaded *Trichoderma which is* identified as *T. viride* before 1969. Therefore, most of the identified taxa before 1969 are in all probability of misidentified since *T. viride* could be a comparatively rare species.

Based on a study, trichoderma harzianum is the bio-control agent especially used in foliar application, seed treatment, as well as soil treatment to protect from various plant pathogens that cause diseases [1-3]. It is also used for manufacturing of enzymes. Currently, the plant diseases caused by *Botrytis, Fusarium* and *Penicillium sp*. are treated by the 3Tac which commercial biotechnological products of Trichoderma. According to research, to meet the increased demands of food and fiber, green revolution is essential in intensified agriculture of near future [4].
In our modern food crop production system, the use of chemical pesticides and fertilizers has adverse effect on natural ecosystem resulted destruction of beneficial organism like honey bees, effect on non-target pests, chemical residues in food, feed and fodder [5]. According to a scholar, *T. harzianum* is mainly used and produced to prevent the development of several soil borne plant pathogens that not only cause diseases but also compete for nutrients and space, do mycoparasitism, produce inhibitory compounds as well as secretes chitinolytic enzymes [6,7].

Trichoderma species are principally green-spore forming ascomycetes present in nearly every type of temperate and tropical soils. Based on a study, most of the *Trichoderma* species are found in decaying plants and within the rhizosphere of plants [8-11]. Their diverse metabolic alteration and aggressive competitive nature help to colonize in their habits.

The antagonistic fungi *T. harzianum* has shown as a promising bio-control agent against soil borne plant pathogens. *T. harzianum* could be recommended not only for control the plant diseases but also as a growth promoter. According to several scholars, bacteria and fungi are multiplied by different fermentation techniques and sold in the different markets as to use for control product growth [12-18]. Study showed the beneficial action of *Trichoderma spp.* is not only fighting for pathogens but also playing role for opportunistic plant symbionts [19-22]. According to previous studies, this interaction with plants initially starts in the rhizosphere and continues to root proliferation, plant growth, and plant protection [23-27]. Hence, these fungi may be applied for redress of impure soil and water by the treatment to become acceptable for plants [28-31]. Based on a study, the disease in Chili could be controlled effectively by *T. harzianum. T. harzianum* are soil borne within the field, green-spore ascomycetes which will be found everywhere in the planet [32-36].

This study is conducting the large-scale production of *Trichoderma harzianum* into the gel/semi gel media and increasing the shelf life, before this study we found only the production of *T. harzianum*is spp. in small amount and short time shelf life.

2. MATERIALS AND METHODS

2.1 The used chemical composition of semi/gel *T. harzinum* for 20L

T. harzinum culture (solid) = 200g, citric acid = 45g, ascorbic acid = 45g, sodium benzoate = 100g, potassium sorbet = 100g, xanthan gum = 200g, food color = 2g to 3g.

2.2 *T. harzinum* culture preparation (solid state fermentation)

The solid-state fermentation of *T. harzinum* is applying for the processes during which insoluble elements or fungi in water are used for growth of microorganism. Within the fermentative process, rice was not exceeding the capability of saturation stage for growth of *T. harzinum*.

Figure 1: Solid & Semi/Gel *T. harzinum*

2.3 Measuring the all ingredients

All ingredients were taken at right amount. The citric acid and ascorbic acid were used to lower pH (4.5 to 4.8) of medium to avoid production of excessive CO2 through fermentation; xanthan gum was added for gel formation and stabilization of the culture and other ingredients.

2.4 Mixing all the ingredients

First the water and xanthan gum were mixed by mixing machine and then the *T. harzianum* culture (solid fermented form) was added and mixed for 30 minutes. Then, all the rest of the ingredients were added and mixed properly for 5 hours for getting homogenized gel, with a shelf life of about 06 (Six) months.

3. RESULTS AND DISCUSSION

T. harzinum was successfully grown in the proposed semisolid medium and showed better result than the other media, (about 5.6 x 10^5 cfu, where 3.56 x 10^3 was in previous media). Semi solid medium has been developed for large scale fermentation of *T. harizanum* inoculums. The newly developed medium showed more cell count of *T. harizanum* and less chemical changes (most probably oxidation) over time compared to solid or liquid media. Two to three days after fermentation, the newly developed semi solid medium showed less production of gas compared to other media.

Table 1: Microbiological result of T. harzinum

Microbiological result of *T. harzinum*				
Previous cfu count of *T. harzinum*	Present cfu count of *T. harzinum* (In this research)	Amount of sample	Sample colour	Remarks
3.56 x 10^3	5.6 x 10^5	1ml	Green & Yellow	Increased the cfu count

Figure 2: After 12 days fermentation at 35°C to 37°C

3.1 Observation and Morphology of *Trichoderma*

This fungus is easy to reproduce at a low-cost cooked rice medium and its potential makes it deserve of the attention of people. The gel formation of *T. harzinum* are developed for the increasing shelf life and prevent the oxidation of liquid suspension of *T. harzinum*, liquid suspension form of *T. harzinum* are oxidized into two or three days and produce CO_2 gas and changes the color of liquid suspension of *T. harzinum.* Before this study,the growth range of *T. harzinum* into a media is only 3.8 x 10^3 cfu, now in this media the growth of *T. harzinum is* more increased and found 5.6 x 10^5 cfu into a normal culture media (Plate Count Agar or Nutrient Agar media).

4. CONCLUSION

This semi/gel solid medium has been developed for large scale fermentation of *T. harizanum* inoculums. The newly developed medium showed more cell count of *T. harizanum* (about 5.6 x 10^5 cfu) and less chemical changes (most probably oxidation) over time compared to solid or liquid media. Two to three days after fermentation, the newly developed semi solid medium showed less production of gas compared to other media. Before this study the growth rate of *T. harzinum* into a media is only 3.8 x 10^3 cfu, now in this media the growth of *T. harzinum is* more increase and found 5.6 x 10^5 cfu into a normal culture media (Plate Count Agar or Nutrient Agar media).

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REFERENCES

[1] Kumar, S., Thakur, M., Rani, A. 2014. Trichoderma: mass production, formulation, quality control, delivery and its scope in commercialization in India for the management of plant diseases. African Journal of Agricultural Research, 9(53), 3838-3852.

[2] Junaid, J.M., Dar, N.A., Bhat, T.A., Bhat, A.H., Bhat, M.A. 2013. Commercial bio-control agents and their mechanism of action in the management of plant pathogens. International Journal of Modern Plant and Animal Sciences, 1(2), 39-57.

[3] Singh, H.B. 2014. Management of plant pathogens with microorganisms. Proceedings of the Indian National Science Academy, 80(2), 443-454.

[4] Pandey, A., Selvakumar, P., Soccol, C., Nigam, P. 1999. Solid State Fermentation for the Production of Industrial Enzymes. Current Science, 77(1),149-162.

[5] Bae, H., Roberts, D.P., Lim, H.S., Strem, M.D., Park, S.C., Ryu, C.M., Melnick, R.L., Bailey, B.A. 2011. Endophytic *Trichoderma* isolates from tropical environments delay disease onset and induce resistance against Phytophthoracapsici in hot pepper using multiple mechanisms. Molwcular Plant Microbes Interaction, 24, 336–51.

[6] Druzhinina, I., Kubicek, C.P. 2005. Species concepts and biodiversity in *Trichoderma* and *Hypocrea*: from aggregate species to species clusters. Journal of Zhejiang University Science, 6(2), 100–112.

[7] Bissett, J. 1991. A revision of the genus *Trichoderma*. III. Section *Pachybasium*". Canadian Journal of Botany, 69(11), 2373–2417.

[8] Samuels, G.J. 2006. *Trichoderma*: Systematics, the Sexual State, and Ecology. Phytopathology, 96(2), 195–206.

[9] Harman, G.E. 2006. Overview of mechanisms and uses of *Trichoderma spp*.". Phytopathology, 96(2), 190–194.

[10] Beyer, W.M, Wuest, P.J., Anderson, M.G. 2007. Green mold of Mushrooms. Pennsylvania State University, Pennsylvania State University extension bulletin, Retrieved 2007-08-02.

[11] Samuels, G.J., Dodd, S.L., Gams, W., Castlebury, L.A., Petrini, O. 2002. *Trichoderma* species associated with the green mold epidemic of commercially grown *Agaricus bisporus*. Mycologia, 94(1), 146–170.

[12] Li, D., Nicosia, M.G., Mosca, S., Mercurio, R., Schena, L. 2015. Dieback of *pinusnigra* seedlings caused by a strain of *Trichoderma viride*. Plant Disease, 99(1), 44–49.

[13] Reason Discovered for the Toxicity of Indoor Mould. 2012. Science Daily, (Oct. 12, 2012): http://www.sciencedaily.com/releases/2012/10/121012074655.htm

[14] Mikkola, R., Andersson, M.A., Kredics, L., Grigoriev, P.A., Sundell, N., Salkinoja-Salonen, M.S. 2012. 20-Residue and 11-residue peptaibols from the fungus *Trichoderma longibrachiatum* are synergistic in forming Na^+/K^+-permeable channels and adverse action towards mammalian cells. FEBS Journal, 279, 4172–4190. doi:10.1111/febs.12010

[15] Dreyfuss, M., Härri, E., Hofmann, H., Kobel, H., Pache, W., Tscherter, H. 1976. Cyclosporin A and C: new metabolites from *Trichoderma polysporum* (Link ex Pers.) Rifai. European Journal of Applied Microbiology, 3, 125–133. doi:10.1007/bf00928431

[16] Chong, F.W., Chakravarthi, S., Nagaraja, H.S., Thanikachalam, P.M., Lee, N. 2009. Expression of transforming growth factor-beta and determination of apoptotic index in histopathological sections for assessment of the effects of Apigenin (4',5',7'-Trihydroxyflavone) on Cyclosporine A induced renal damage. Malaysian Journal of Pathology, 31(1), 35–43.

[17] Azin, M., Moravej, R., Zareh, D. 2007. Self-directing optimization of parameters for extracellular chitinase production by *Trichoderma harzianum* in batch mode. Process Biochemistry, 34(6–7), 563–566. doi:10.1016/S0032-9592(98)00128-9

[18] Felse, P.A., Panda, T. 1999. Production of xylanase by *Trichoderma longibrachiatum* on a mixture of wheat bran and wheat straw: Optimization of culture condition by Taguchi method. Enzyme and Microbial Technology, 40(4), 801–805. doi:10.1016/j.enzmictec.2006.06.013

[19] Tan, S.H. 2013. Morphological characterization and sequence analysis of 5.8s-its region of *Trichoderma* species. Bachelor Thesis, Faculty of Science, Universiti Tunku Abdul Rahman. Available online: http://eprints.utar.edu.my/844/

[20] Olabiyi, T.I., Ojo, O.J., Adewuyi, B.O. 2016. Impact assessment of neem compost and *Trichoderma harzianum* solution in the control of root knot nematode disease on cowpea. International Journal of Phytopathology, 5(2), 67-71.

[21] Pratibha, S., Amr, N.P., Mahesh, K.S., Swati, D. 2012. Field demonstration of *Trichoderma harzianum* as a plant growth promoter in wheat (*Triticum aestivum* L). Journal of Agricultural Science, 4(8), 2012.

[22] Jaime, M., Luis, V., Rodrigo, H. Ximena, B. Luz, M.P. 2009. Biocontrol capacity of wild and mutant *Trichoderma harzianum* (Rifai) strains on *Rhizoctonia solani* 618: effect of temperature and soil type during storage. Electronic Journal of Biotechnology, 12(4), 2314-2321.

[23] Maria, H.S.G., Gustavo, H.G. 1998. *Trichoderma harzianum* transformant has high extracellular alkaline proteinase expression during specific mycoparasitic interactions. Genetics and Molecular Biology, 21(3). DOI: 10.1590/S1415-4757199800030000.

[24] Maria, H.S.G., Gustavo, H.G. 1999. *Trichoderma harzianum* transformant has high extracellular alkaline proteinase expression during specific mycoparasitic interactions. Genetics and Molecular Biology, 23(6), 1641-1653.

[25] Ashraf, A.K., Sinha, A.P., Rathi, Y.P.S. 2005. Plant growth promoting activity of *Trichoderma harzianum* on rice seed germination and seedling vigor. Indian Journal of Agricultural Research, 39(4), 256-262.

[26] Nihad,H.M., Jamal, H.K. 2014. Effect of two isolates of *Trichoderma harzianum* on total nitrogen, chlorophyll a & b contents and yield of wheat (*Triticum aestivum* L) class Eba'a- 95. International Journal of Science and Research, 3(8), 2319-7064.

[27] Laila, N., Seri, I.B.M., Noorhazira, B.S. 2015. *Trichoderma harzianum* T32 growth and antagonistic performance against *Ganoderma boninense* on different culture media. 3rd International Conference on Biological, Chemical & Environmental Sciences (BCES-2015) Sept. 21-22, 2015 Kuala Lumpur (Malaysia), Available online: umkeprints.umk.edu.my/5546/1/2428C0915047.pdf.

[28] Naeimi, S., Okhovvat, S.M., Javan-Nikkhah, M., Vágvölgyi, C., Khosravi, V., Kredics, L. 2010. Biological control of *Rhizoctonia solani* AG1-1A, the causal agent of rice sheath blight with *Trichoderma* strains. Phytopathologia Mediterranea, 49(3), 287-300.

[29] Benitez, T., Rincin, A.M., Limin, M.C. Codin, A.C. 2004. Biocontrol mechanisms of *Trichoderma* strains. International Microbiology, 7, 249-260.

[30] Elad, Y. 1994. Biological control of grape grey mould by *Trichoderma harzianum.* Crop Protection, 13, 35-38.

[31] Fourie, P.H., Halleen, F., van der Vyver, J., and Schreuder, W. 2001. Effect of *Trichoderma* treatments on the occurrence of decline pathogens in the roots and rootstocks of nursery grapevines. Phytopathologia Mediterranea, 40, 473-478.

[32] Fourie, P.H., Halleen, F. 2002. Investigation on the occurrence of *Phaeomoniella chlamydospora* in canes of rootstock mother vines. Australian Plant Pathology, 31, 425-426.

[33] Fourie, P.H., Halleen, F. 2004. Occurrence of grapevine trunk disease pathogens in rootstock mother plants in South Africa. Australasian Plant Pathology, 33, 313-315.

[34] Fourie, P.H., Halleen, F. 2004. Proactive control of Petri disease of grapevines through treatment of propagation material. Plant Disease, 88, 1241-1245.

[35] Fourie, P.H., Halleen, F. 2006. Chemical and biological protection of grapevine propagation material from trunk disease pathogens. European Journal of Plant Pathology, 116, 255-265.

[36] Halleen, F., Crous, P.W., Petrini, O. 2003. Fungi associated with healthy grapevine cuttings in nurseries, with special reference to pathogens involved in the decline of young vines. Australasian Plant Pathology, 32, 47-52.