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Evaluation of Different Concentration of Jaggery (as Cheaper Carbon Source) for Growth and Sporulation of *Trichoderma harzianum* (Th14)

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ABSTRACT

Among the tested different concentration (2, 4, 6, and 8%) of jaggery for the growth and sporulation of *T. harzianum* (Th14) maximum biomass(mg/100ml) and sporulation (spores/ ml) was observed at 8 per cent jaggery (555mg; 5.00x10^8) and was at par with 6 per cent jaggery (512mg; 4.33x10^8). However, maximum biomass was observed in PDB (789mg; 5.42x10^8) and was at par with jaggery 8 per cent in sporulation. Minimum biomass and sporulation was observed with potato broth (229; 2.75x10^6) i.e. without any carbon source. Present investigation revealed that jaggery could be exploited as a cheaper substrate for the mass production of *Trichoderma* for the management of plant diseases under sustainable agriculture.

Key words *Jaggery, Growth and Sporulation, Trichoderma harzianum*

Every living organism requires food for the growth and reproduction; fungi are not exception to it. Fungi secure food from the substrates upon which they live in. All media are not equally good for the growth and sporulation of all fungi. Faster and luxuriant growth of fungi can only be obtained when grown on suitable substrate. Locally available and agricultural wastes/products have been found to be excellent substrates for on farm production of antagonists. The substrates used for the mass production of fungal antagonist through solid state fermentation and liquid fermentation were reported by various workers (Elad, *et al*., 1980; Papavizas, *et al*., 1984; Upadhyay and Mukhopadhyay, 1986; Harman, *et al*., 1991; Jahan, *et al.,* 2013 and Faruk, *et al.*, 2014). For the commercialization of the biocontrol it is necessary to produce maximum quality biomass with least economic cost. Various liquid substrates like sugarcane baggase, fruit juice waste, vegetable waste, rotten wheat grains etc. are being used for mass multiplication of *T. harzianum* with various degree of success. Therefore, looking towards need for large scale cost effective production of microbial pesticides.

The knowledge of nutritional requirements essential for the successful cultivation of microorganisms like carbon, nitrogen, aminoacids, vitamins. The effect of the nutrient sources on the growth and development of microorganisms is studied by Gao *et al.,* 2007. The medium and their contents are responsible for the mycelial growth and spore yield of a specific microorganism. Variation in the type of carbon and nitrogen sources besides changes in pH, temperature, incubation period etc. can greatly influence mass production of antagonistic fungi (Thakur, *et al.,* 2009). Present study was carried out and evaluated locally available substrate jaggery (as cheaper carbon source) for mass multiplication of *T. harzianum* (Th14).

MATERIALS AND METHODS

An experiment was conducted in the Oilseed Pathology Lab of the Department of Plant Pathology, G.B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand) India to study effect of different concentrations (2, 4, 6, and 8%) of jaggery on growth and sporulation of *T. harzianum* (Th 14).

The *Trichoderma harzianum* isolate Th14 obtained from culture collections of Biocontrol laboratory, Department of Plant Pathology, GBPUA&T, Pantnagar for the present investigation. The fungal antagonist was maintained on potato dextrose agar (PDA) slant and stored in refrigerator for further studies.

Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) were prepared following the standard procedure (Anonymous, 1968). After preparation of media, pH of the medium was adjusted to 6.00 by adding adding N/10, HCl/ NaOH using pH meter. Prepared medium was sterilized in an autoclave at 121°C temperature for 20 minutes. The flasks (250 ml capacity) containing 100 ml of each sterilized liquid medium viz. Jaggery (2, 4, 6,

Table 1. Evaluation of different concentrations of Jaggery (as cheaper carbon source) on mycelial growth and sporulation of *Trichoderma harzianum*

Treatment	Cultural characters	Dry mycelial weight* (mg/100ml)	Spores/ ml* (x10^6)
Jaggery 2%	Thin mycelial mat with slight sporulation	339^b	325^b
Jaggery 4%	Thick mycelial mat with dark green sporulation	456^c	403^c
Jaggery 6%	Thick mycelia mat with good dark green sporulation	512^d	433^{cd}
Jaggery 8%	Thick mycelial mat with good dark green sporulation	555^d	500de
PDB	Thick mycelial mat with good dark green sporulation	789^e	542^e
PB	Thin mycelial mat with slight sporulation	229^a	2.75^a
CD(0.05)		47	73
CV (%)		5.48	11.10

*Mean of three replicates

Values in each vertical column followed by same letter do not differ significantly

and 8%), PDB and PB were inoculated with 5 mm discs (2 No.) cut from 4 days old actively growing culture of *T. harzianum* (Th14). The flasks were plugged in aseptic conditions and placed in incubator at 27±1^{0}C for 12 days. Three replications were kept for each medium.

Determination of mycelial biomass and sporulation

After 12 days incubation (DAI) the fungal mycelial mass in each flask of each treatments were separated by Whatman paper No.1. The fungal bio biomass on the filter paper was dried at room temperature for 48 hours. Then the dry weight (mg/ 100ml) was measured with electronic balance. The mycelia mat along with spores was collected from each flask (100 ml broth) on blotter paper. The mycelial mat was air dried and make it in fine powder. The powder obtained was properly mixed in their respective culture broth. Take 1ml of *Trichoderma* from well shake culture broth (in which spore of mycelial powder was mixed) it in suspended in 10 ml of sterilized distilled water, stirred well. One ml of this suspension, well shaken, was added to 9 ml of sterilized distilled water to make 10^{-1} dilution. The procedure was repeated till the desired dilutions were obtained. The spore concentration was measured using haemocytometer. Final count of spore/ ml was calculated using following formula:

Spores/ ml= n x 25 x10^4x diluation factor

N= average no. of spore in medium square of haemocytometer (0.2x0.2x.1mm^3)

Statistical design

The experiment was conducted with completely randomized design (CRD) and the experimental data were statistical analysied using STPR1&2. Data were subjected to analysis of variance and treatment means were compared by an appropriate Duncan's multiple range test ($P < 0.05$) under SPSS 16.

RESULTS AND DISCUSSION

Results indicated (Table1) that among the tested different concentrations of jaggery for the growth and sporulation of *T. harzianum,* significantly maximum biomass production was observed in PDB (789g/100ml) followed by jaggery 8% (555g/100ml) and jaggery 6% (512mg/100ml) and both were at par to each other. However, Jaggery 4% (403mg/100ml) produced significantly higher biomass compare to jaggery 2% (329mg/100ml) and potato broth (229mg/100ml) while both were also at par with each other on biomass production. In term of spore production, maximum sporulation was observed in PDB (5.42x10^8/ml) and was at par with jaggery 8% (5.00x10^8/ml). There was no significance observed between Jaggery 8% & Jaggery 6% (4.33x10^8/ml) and jaggery 4%

($4.23x10^8$/ml) & Jaggery 6%. Jaggery 2% ($3.25x10^8$/ml) was produced significantly higher sporulation as compare to potato broth ($2.75x10^6$/ml). Jahan *et al.,* 2013 studied the fresh weight and dry weight of *T. harzianum* on different media and found PDB as the best, which was agreement to our findings. Our observations also related with that reported by Chaudhari *et al.,* 2011 who observed the highest CFU on solid sugarcane baggase media ($91.3x10^8$) and on liquid media of sugarcane baggase ($84.7x10^8$). Prasad *et al.,* 2002 also reported that Jaggery (3%) and wheat flour (10%) enhance conidial yield of *T. harzianum*. Various substrates like sugarcane baggase, fruit juice waste, vegetable waste, rotten wheat grains etc. are being used for mass multiplication of *T. viride* with various degree of success (Esposito and Silva, 1998).

Biomass and sporulation of *T. harzianum (*Th 14) was influenced by jaggery concentrations. Biomass and sporulation increased with increases jaggery concentration. Maximum biomass and sporulation was observed at jaggery 8 per cent while minimum at Jaggery 2 per cent. The high spore count of the tested fungus on jaggery may be possibly due to high carbohydrate content in them. However, in case of tested fungus, the lower spore count was observed on lower concentration in comparison with control (PB) might be due to lower carbohydrate content present in jaggery. The result of the present study revealed the suitability of jaggery as substrate material for the commercial preparation of *Trichoderma* formulation and could be exploited as a cheaper substrate or as supplements for the better growth and sporulation of *Trichoderma* with some amendments

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LITERATURE CITED

Anonymous, 1968. Plant Pathologist's Pocket Book. Commonwealth Mycological Institute, pp: 394-395.

Chaudhari, P. J., Shrivastava, P. and Khadse, A. C. 2011. Substrate evaluation for mass cultivation of *Trichoderma viride*, *Asiatic J. Biotech Res.* **2** (4):441-446

Elad, Y., Chet, I. and Katan, J. 1980. *Trichoderma harzianum*: A biocontrol agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. *Phytopathology,* **70**: 119-121.

Esposito, E. and M. da Silva, 1998. Systematics and environmental application of the genus *Trichoderma*. *Crit. Rev. Microbiol.*, **24**: 89-98.

Faruk, M. I., Rahman, M. L., Islam, M. N., Rahman, M. M. and Rahman, M. A. 2014. Assessment of carrier materials to formulate *Trichoderma harzianum* bio-fungicide for controlling foot and root rot disease of brinjal in seed bed. *Agricultural Science*, **2** (2): 21-30.

Gao, K., Liu, X., Kang, Z. and Mendgen, K. 2005. Mycoparasitism of *Rhizoctonia solani* by endophytic *Chaetomium spirale* ND 35: Ultrastructure and cytochemistry of the interaction. *Journal of Phytopathology*. **153**:280-290.

Harman, G.E., Jin, X., Stasz, T.E., Peruzzaoil, G., Leopoid, A.C. and Taylor, A.G. 1991. Production of conidial biomass of *Trichoderma harzianum* for biological control. *Biological control,* **1:**23-38.

Jahan, N., Sultana, S., Adhikary, S. K. and Rahman, S. 2013. Evaluation Of The Growth Performance Of *Trichoderma harzianum* (Rifai.) On Different Culture Media. *Journal of Agriculture and Veterinary Science,* **3**(4): 44-50

Papavizas, G.C., Dunn, M.T., Lewis, J.A. and Beagle-Ristaino, J. 1984. Liquid fermentation technology for experimental production of biocontrol fungi, *Phytopathology*, 1171-1175.

Prasad, R D., Rangeshwaran, R. and Sunanda, C. R. 2002. Jaggery – an easily available alternative to molasses for mass production of *Trichoderma harzianum*. *Plant Disease Research* **17:** 363–365.

Thakur, D., Bora, T. C., Bordoloi, G. N. and Mazumdar, S. 2009. Influence of nutrition and culturing conditions for optimum growth and antimicrobial metabolite production by *Streptomyces*sp. 201. *Journal Medical Mycology,* **19**:161-167.

Upadhyay, J.P. and Mukhopadhyay, A.N. 1986. Biological control of *Sclerotium rolfsii* by *Trichoderma harzianum* in sugarbeet. *Tropical Pest Management,* **32**: 215-220.

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