Optical density - a tool for the estimation of spore count of *Trichoderma viride*

R. R. Waghunde*, Priya John, B. M. Naik, K. U. Solanky and A. N. Sabalpara

ABSTRACT

Spore count is an important parameter in mass multiplication of entomopathogens including *Trichoderma viride* for quality analysis. In the present investigation, it has been found that optical density (OD) is an easy and reliable method as compared to haemocytometer count (HC) reading. A positive correlation between *T. viride* concentration (0.05 to 0.5%) and OD had been found in proportional to HC count at seventh day and increased at 10th day after inoculation on sugarcane juice medium (SJM). The spore count of *T. viride* can be determined at 550nm with spectrophotometer in place of haemocytometer by measuring optical density.

Key words : Spectrophotometer, haemocytometer, *T. viride*, spore count, tool

INTRODUCTION

Trichoderma spp. have been widely used as antagonistic fungal agents against several pests as well as plant growth enhancers. Faster metabolic rates, anti microbial metabolite and physiological conformation are key factors which chiefly contribute to the antagonism of these fungi. Spore produced by the microorganism acts as the reproductive unit and the spore count of biological control agent indicates its potency of antagonism against the pathogen. Several methods have been developed to quantify sporulation by haemocytometer (Singh and Singh, 2007). But one of the most common ways used in plant pathology is the use of the haemocytometer (Valdez and Piccalo, 2007). Spore suspension is widely used as inoculum in phytopathological experiments as a mother culture for mass multiplication of *Trichoderma* spp., *Pseudomonas* spp., *Bacillus subtilis* and *Verticillium lecani* (Kulkarni and Sagar, 2007; Ashnaei *et al.*, 2009). Spore count is an essential quality parameter for bioagent efficacy. But the use of haemocytometer is tedious and time consuming and unreliable. Different workers used optical density (OD) to quantify the spore count. Valdez and Piccalo (2007) used OD as a tool to quantify *Penicillium alli* at 340nm wavelength. Asher *et al.* (2007) measured spore count of powdery mildew, yellow rust and brown rust of barley at 1900nm, 2252nm and 2308nm wavelength, respectively. Kraus *et al*. (2004) studied that physiological character such as spore and conidiophores of *Trichoderma brevicompactum* were studied at 490nm.The present study was carried out on *Trichoderma viride* which is used as an important biopesticide against soil borne pathogens such as *Fusarium solani*, *F. moniliformae*, *Machrophomina phaseolina* and *Aspergillus niger*. *T.viride* as a bioagent was massively used for the production and mass multiplication. Hence, an attempt was made to use spectrometric tool for the quantification of *Trichoderma viride* spore.

MATERIALS AND METHODS

An experiment was conducted to compare the spore count and optical density for the quantification of *T. viride* by spectrophotometer. *Trichoderma viride* was isolated from sugarcane rhizosphere. The culture was grown on 10% sugarcane juice medium (SJM) and potato dextrose broth (PDB) at room temperature. The sporulation and mycelial growth were more in SJM as compared to potato dextrose broth. As *T. viride* showed minimum sporulation on 7th and maximum sporulation on 10th day. Therefore, 7 and 10 day old cultures of *T. viride* were used for the preparation of stock suspension to check any variation on spore count. The spore were separated with the help of sterilized fine paint brush and weighed for different concentration such as 0.05g, 0.1g, 0.2g, 0.3g, 0.4g and 0.5g. The mycelial mat was separated by filtering the spore suspension through the sterilized muslin cloth (Trinci, 1972). The stock suspension were kept on Rotary Flask Shaker (MAC, MSW-301, New Delhi) for 30 min, 3ml of the suspension added in the cuvetts having 10mm light path (Type No.100-600QG Hellma). The equipment was calibrated with 3ml of the blank solution (SJ medium). The suspension was

Table 1. Spore suspension optical density (OD) and spore count (HC) of *Trichoderma viride* on seventh and tenth day after inoculation

Spore Concentration (%)	7th Day			10th Day		
	% Transmission	Absorbance	HC (10^6 spore / ml)	% Transmission	Absorbance	HC (10^6 spore / ml)
0.05	96.8	0.014	0.01	85	0.071	1.92
0.1	95.6	0.020	1.46	77.3	0.112	6.76
0.2	70.5	0.152	5.70	71.3	0.147	5.68
0.3	68.3	0.166	6.20	65.6	0.183	7.56
0.4	60.5	0.218	8.30	61.7	0.210	8.24
0.5	54.0	0.268	9.80	54.4	0.264	9.72
R^2		0.91	0.92		0.98	0.77

Optical density was measured at 550 nm

screened for 100%T at 400 to 800nm wavelength under Double Beam UV-VIS spectrophotometer (Mod. No. 5704SS). An OD value for 0.05 to 0.5% at 550nm was fixed. Corresponding haemocytometer (HC) readings were also taken using Fuchs Rosenthal Slide (Singh and Singh, 2007). Each concentration was replicated three times to obtain a satisfactory spore suspension. The data were analyzed by regression analysis.

RESULTS

For preliminary analyses we tested the wavelength from 400 to 800nm for 100%T of the SJ medium. The 100% T was observed in the case of wavelength ranging from 530 to 570nm for SJ medium and 550nm wavelength was considered for further study. The 0.05 to 0.5% concentrations of conidia showed more than 40%Transmission. The sensitivity of method reduced when concentration of the conidia were out of this range. 0.05 to 0.5g suspensions showed 0.014 and 0.071, 0.020 and 0.112, 0.152 and 0.147, 0.166 and 0.183 and 0.268 and 0.264 OD and HC reading at 7th day and 10th day, respectively (Table 1). With the increase in the concentration of *T. viride* from 0.05 to 0.5%, the OD, HC also increased. To check the variability in spore count, OD value and HC was determined both on 7th and 10th day. There was not much difference in the OD and HC values on the 7th day. Whereas, on 10th day the spore count differed quite higher both in OD (R^2 = 0.98) and HC (R^2 = 0.77). This evidence provides that optical density is accurate in determining spore count.

DISCUSSION

It has been found that several workers used spectrophotometry for spore count. This is first instance that *T. viride* spore count is determined by spectrophotometer. In present work, the spore count of bioagent *T. viride* could be determined at 550nm with corresponding haemocytometer reading. During the present study it was observed that OD is directly proportional to HC reading. Hence, it can be used as tool to quantify the spores of different bioagents. The process is quick and highly reliable to detect the spore count. It was found that with increase in concentration of spore, there is an increase in absorption value as well as the spore count. Wells and Spalding (1975) also used 550nm wavelength for *Geotrichum candidum* to study correlation of OD value of spectrophotometer with HC reading. Asher *et al.* (2007) noted spore count of powdery mildew, yellow rust and brown rust of barley at 1900, 2252 and 2308nm. Tsai and Erwin (1975) found 37 µm sclerotia of *Verticillum albocitrum* at 700nm. Wells and Spalding (1975) showed direct relationship between OD and HC reading at 550nm for *Geotrichum candidum*. Optical density can be used to determine the spore count of bioagent. It is accurate and reliable and it takes less time.

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