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Evaluation of local solid and liquid substrates for growth and sporulation of *Trichoderma asperellum*

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Abstract
Rotten maize grain, maize stone and banana pulp supplemented with jaggery were evaluated as substrates for the growth and sporulation of *Trichoderma asperellum*. Significant increased sporulation per gram was observed in jaggery added substrate. Among solid substrates, significant maximum growth and sporulation per gram was observed in Sorghum grain+2% Jaggery ($8.31x10^8$) followed by Maize grain+2% Jaggery ($5.54x10^8$) and Sorghum grain ($5.24x10^8$). Different concentrations (2, 4, and 6%) of Banana pulp in water was also evaluated and maximum sporulation (spores/ ml) was observed in potato pulp+ 2%Jaggery ($10.20x10^7$) followed by Banana pulp (6%) + 2%Jaggery ($9.72x10^7$) and was at par with Banana pulp (4%)+2%Jaggery. Minimum sporulation was observed with Banana pulp (2%) + ($3.45x10^7$). The agriculture waste like rotten maize and banana supplemented with jaggery may be utilized for the mass production of *Trichoderma asperellum* bioagent.

Keywords: *Trichoderma asperellum*, rotten maize, banana pulp, substrate and sporulation

Introduction
Trichoderma is most popular biocontrol agent against several plant pathogenic fungi throughout the world and the effectiveness of *Trichoderma* spp. against several plant diseases has been reported by several researchers (Srivastava *et al.*, 2008; Mairzano *et al*., 2013; Singh *et al*., 2013;, Rai and Maurya, 2021) [1, 2, 3, 4]. Every living organism requires food for the growth and reproduction; *Trichoderma* are not exception to it. *Trichoderma* 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. The selection of the cheapest and easily available substrates which favours the growth and sporulation of *Trichoderma* is currently most important researchable issue. 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; Faruk, *et al.*, 2014) [5, 6, 7, 8, 9]. For the commercialization of the biocontrol it is necessary to produce maximum quality biomass with least economic cost. Production of adequate quantities of good quality inoculum is an essential component of the biocontrol programme.

Converting agricultural waste like rotten maize, maize stone, banana fruit in to value added biopesticides to replace chemical pesticides for plant protection is good alternative for environmental sustainability and resource recycling. Huge amount of solid waste like rotten maize grain, banana, wheat, sugarcane baggase, fruit juice wastes, vegetable waste increasing pollution and disposal problems but farmers can get additional income by mass production of *Trichoderma* on it. For mass multiplication of bioagent through solid state fermentation technology an enormous quantity of spore biomass is needed. Various substrates like sugarcane baggase, fruit juice waste, vegetable waste, rotten wheat grains etc. are being used for mass multiplication of *Trichoderma viride* with various degree of success. (Babu and Pallavi, 2013) [10]. Present investigation is carried out to evaluate locally available rotten grain of maize supplemented with jaggery for growth and sporulation of *Trichoderma asperellum* for sustainable environment and sustainable agriculture.

Materials and Methods
The research was carried out in the Bio-control Lab, Department of Plant Pathology, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar.

The *Trichoderma asperellum* strain was obtained from Bio-control Lab, from the University for the present investigation. The fungal antagonist was maintained on potato dextrose agar (PDA) slant and stored in refrigerator for further studies.

Selection of substrates

Locally availably substrates namely Maize, Sorghum, Maize stone and Banana pulp supplemented with jaggery were evaluated for the growth and multiplication of *Trichoderma asperellum.*

Evaluation of Solid Substrates

The flasks (250 ml capacity) containing 100g of each sterilized broken maize grain, sorghum and maize stone alone and supplemented with jaggery (2%) sterilized in an autoclave at 121 °C temperature for 20 minutes. After sterilization, substrates were inoculated with 5 mm discs (2 No.) cut from 4 days old actively growing culture of *T. asperellum*. The flasks were plugged in aseptic conditions and placed in incubator at 27±1 °C for 7 days. Three replications were kept for each treatment.

Evaluation of different concentrations of Banana pulp

The flasks (250 ml capacity) containing 100 ml of water (added with 2%, 4% and 6% banana pulp) supplemented with jaggery (2%) sterilized in an autoclave at 121 °C temperature for 20 minutes. After sterilization, medium were inoculated with 5 mm discs (2 No.) cut from 4 days old actively growing culture of *T. asperellum*. The flasks were plugged in aseptic conditions and placed in incubator at 27±1 °C for 7 days. Three replications were kept for each medium.

Determination of Growth and Sporulation

After 7 days incubation (DAI) the *Trichoderma* growth character in each flask of each treatments were visually recorded and colonized substrates air dried and make it in fine powder. Final count of spore/ g was measured by haemocytometer. In evaluation of banana pulp experiment, *Trichoderma* growth characters in each flask of each treatment were visually recorded and mycelial mat properly mixed in their respective culture broth. Final count of spore/ ml was measured by haemocytometer. Taken 1ml 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 OPSTAT.

Results and Discussion

Evaluation of solid substrates

Sorghum grain, maize grain and maize stone supplemented with 2 per cent jaggery enhanced the growth and sporulation of *Trichoderma asperellum*. Thick mycelial mat with good sporulation observed on sorghum and maize subtracts (Fig.1). Data presented in Table 1 revealed that significant maximum sporulation per gram was observed in Sorghum grain+2% Jaggery (8.31x10^8) followed by Maize grain+2% Jaggery (5.54x10^8) and Sorghum grain (5.24x10^8). Minimum sporulation was observed in Maize stone (6.38x10^6). The present findings are in accordance with the findings of Singh *et al.* (2012) [11] who observed maximum in sorghum grains (132x10^7) compared to wheat flour and other substrates. Kousalya Gangadharan (1990) [12] also reported that sand maize media, sorghum grain medium were useful for mass multiplication of *T. harzianum.* Prasad *et al.* (2002) [13] reported that Jaggery (3%) supplemented in wheat enhanced conidial yield of *T. harzianum*.

Evaluation of banana pulp supplemented with jaggery

Banana is also a good source of carbohydrate and vitamins which favours the growth and sporulation of *Trichoderma*. Rotten banana pulp was used for the study. Banana pulp was evaluated at different concentrations (2, 4, and 6%) in 100 ml of water supplemented with jaggery for the sporulation of *Trichoderma* and maximum sporulation (spores/ ml) was observed in potato pulp+ 2%Jaggery (10.20x10^7) followed by Banana pulp (6%)+ 2%Jaggery (9.72x10^7) and was at par with Banana pulp (4%)+2%Jaggery (Table 2 & Fig.2). Minimum sporulation was observed with Banana pulp (2%) + (3.45x10^7). Sinha *et al.* (2021) [14] evaluated biowastes of mango, carrot, papaya, banana, chukandar, pomegranate, orange, mosambi and maximum number of spores/ml of *T. harzianum* were obtained on biowaste of carrot followed by mango, chukandar, banana, papaya, orange and mosambi. Zhang *et al.* (2022) [15] also evaluated cassava peels, banana pseudostem, coconut shell, sugarcane bagasse, and pineapple peels for the rapid production of biopesticide *Trichoderma* Brev T069 and maximum spore yield of 9.31 × 10^9 spores/g was observed in cassava peel.

Table 1: Evaluation of solid substrates supplemented with jaggery for growth and sporulation of *Trichoderma asperellum*

Medium	Growth Characters	Sporulation (x10^6 spores/g)	Spores/g
	5 DAI*	7DAI*	
Sorghum	Thick mycelial growth and dark green sporulation	523.75	5.24x10^8
Maize	Thick mycelial growth with green sporulation	323.75	3.24x10^8
Maize stone	Thiin mycelial growth and light green sporulation	6.38	6.38x10^6
Sorghum+2%jaggery	Thick mycelial mat and with good dark green sporulation	831.25	8.31x10^8
Maize +2%jaggery	Thick mycelial mat with dark green sporulation	553.75	5.54x10^8
Maize stone+ 2% jaggery	Thin mycelial growth with light green sporulation	13.50	1.35x10^7
CD (0.05)		55.95	
CV (%)		10.03	

Table 2: Evaluation of banana pulp supplemented with jaggery for growth and sporulation of *Trichoderma asperellum*

Medium	Growth Characters	Sporulation ($x10^6$ spores/ml)	Spores/ml
	5 DAI*	7DAI*	
Banana pulp (2%)	Thin mycelial growth and light sporulation	34.50	$3.45x10^7$
Banana pulp (4%)	Thick mycelial mat with light green sporulation	52.83	$5.28x10^7$
Banana pulp (6%)	Thick mycelial mat with green sporulation	59.33	$5.93x10^7$
Banana pulp (2%)+2%jaggery	Thick mycelial mat with green sporulation	71.50	$7.15x10^7$
Banana pulp (4%)+2%jaggery	Thick mycelial mat with good dark green sporulation	89.00	$8.90x10^7$
Banana pulp (6%)+2%jaggery	Thick mycelial mat with good dark green sporulation	97.20	$9.72x10^7$
Potato pulp (2%)	Thick mycelial mat with l dark green sporulation	54.33	$5.43x10^7$
Potato pulp (2%) + 2% Jaggery	Thick mycelial mat with good dark green sporulation	102.00	$10.20x10^8$
CD (0.05)		10.10	
CV (%)		8.29	

Fig 1: Evaluation of solid substrates supplemented with jaggery for growth and sporulation of *Trichoderma asperellum*

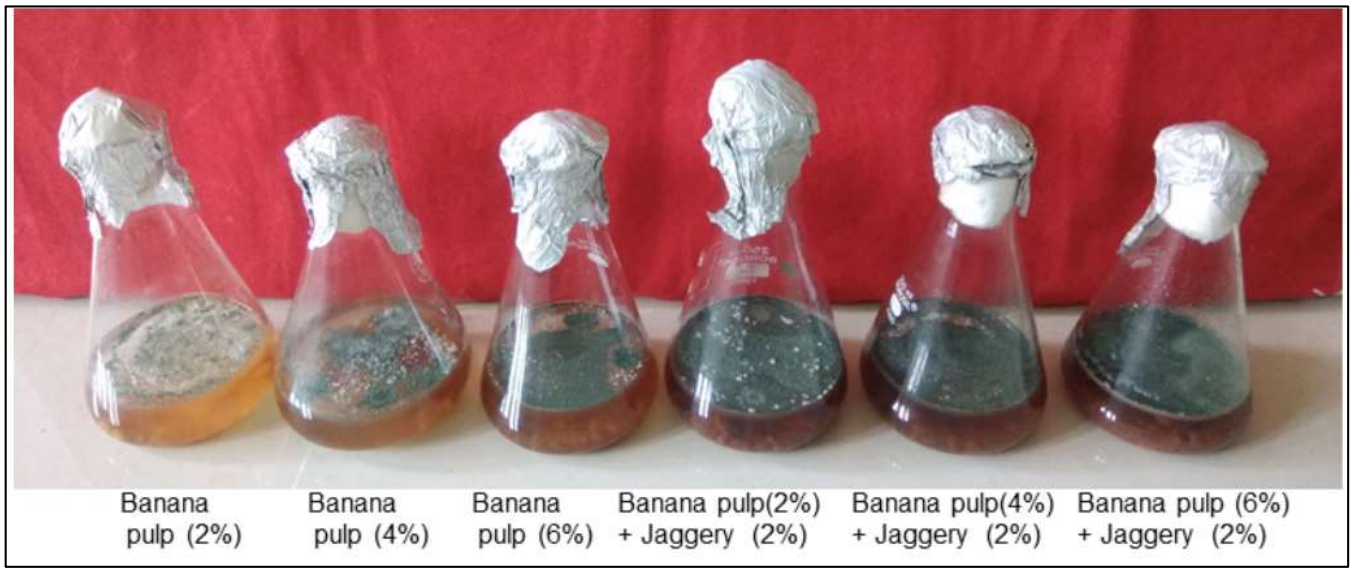

Fig 2: Evaluation of banana pulp supplemented with jaggery for growth and sporulation of *Trichoderma asperellum*

Conclusion

The result of study indicate that locally available waste viz. rotten maize and banana has got immense potential for growth and sporulation of *Trichoderma asperellum* which can be used for mass production of *Trichoderma* or in agriculture field for the management of soil borne diseases.

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