

Screening of Some New Substrate for Mass Multiplication of *Trichoderma harzianum* and *Pseudomonas fluorescens* and Determination of Suitable Moisture Level

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Abstract: Various low-cost agricultural waste products were screened for mass multiplication of *T. harzianum* and *Pseudomonas fluorescens* under normal storage condition and most effective formulated product of *T. harzianum* and *Pseudomonas fluorescens* was evaluated *in vitro*. Among different substrate including some of farm wastes and residues *viz.* wheat straw, wheat straw + rice husk, rice straw, sugarcane bagasse, sugarcane pressmud, bajra grains + wheat straw, sorghum grains + rice husk, chick pea flour + wheat straw, rotten cow dung + unrotten cow dung. The sorghum grains + rice husk was best growth substrate for *T. harzianum* and chickpea flour + wheat straw was best substrate for *P. fluorescens*. 70 per cent moisture was appropriate for the growth and mass multiplication of both the antagonist.

Keywords: Biocontrol agents, Moisture, Substrate.

INTRODUCTION

Biocontrol agent for commercial development is the availability of a cost effective production of a stable formulated products that sustained growth of the antagonist and their storage under normal condition as well as with a novel delivery system (Frave1 2005). Most of the commercial formulations of *Trichoderma* are based on inert material (talc), act only as carrier and bulking material but do not support the growth and survival of antagonists under storage condition. Formulation based on the agricultural waste products, *viz* bran of grains, oil cakes, farmyard manure etc are found to support excellent growth carrier and storage media (Patibanda *et al.* 2003). Besides the nature and properties of organic wastes, different physical factors like temperature, moisture content and P^H of the

substrate are considered to be the important determinants for the mass multiplication of the *T. harzianum*. The species of *Trichoderma* typical to cool geographic regions. possess lower temperature optimum than the species from warm climatic regions (Reddy and Khowles *et.al.* 1965). The growth and sporulation of *Trichoderma harzianum* has been reported at high in the substrate at 30°C than 20°C (Bundopadhyay *et.al* 2003). Moisture content of substrate is another key factor in determining the growth of *Trichoderma spp.* As these species arc neither able to tolerate high moisture level nor survive under deficit of moisture in natural ecosystems. *T.harzianum* grew and sporulated better when the moisture content of wheat bran + saw dust was 35 percent (Elad *el al.*, 1980).

MATERIALS AND METHODS

Preparation of TSM

All the ingredients were mixed with distilled water; agar was added to it in a stainless steel pan and stirred with glass rod for proper mixing. Now the medium was filtered through a muslin cloth by squeezing out whole liquid. 200 ml dissolved medium was placed in each 500 ml capacity flask. Flasks were tightly plugged with non-absorbent cotton and wrapped with butted paper and rubber band. Medium was autoclaved at 1.1 kg/cm² pressure for 20 minutes (121.6°C) and cooled before pouring in to Petri plates.

Preparation of King's(B) medium

Medium was prepared following the method used for preparing TSM. The Bacteria initially isolated in a pure culture on King's B medium and sub cultured on PDA slants. After initial growth of *P.fluorescens* on King's B medium, identity of the bacterium was confirmed following standard. After proper growth, bacteria culture was stored in a refrigerator for long term storage, so as to be used in further studies.

Collection of Substrate

The materials were collected from local area, village, sugar factory and local market. These aforementioned substrate were used after adding additional water to maintain different moisture regimes as mentioned earlier. For this purpose substrate were mixed with sterilized distilled water (SDW) and autoclave at 121.6° C (1.1 kg/cm²) for 20 minutes. The flasks were allowed to cool at room temperature prior to inoculation. Flasks containing substrates were inoculated with 3-4 days old actively growing culture of *Trichoderma* spp. and *Pseudomonas* spp. (2-3 bits of about 5 mm size) under aseptic condition in laminar flow as per experiment need.

The flasks were shaken thoroughly once a day, and incubated at 28± 2° C for 30 days.

For each treatments, three replicates of flasks were maintained. Population dynamics of both the bioagents *i.e.* *Trichoderma harzianum* and *Pseudomonas fluorescens* under different moisture regimes were determined by following serial dilution plate technique, after 10, 15 and 20 days of inoculation.

Substrates assessment and maintenance of different level of moisture

Each substrates (*i.e.* sorghum grains + rice husk, bajra grains + wheat straw, sugarcane pressmud, sugarcane bagasse, rice straw, chickpea flour + wheat straw, wheat straw, wheat straw + rice husk and rotten cow dung + unrotten cow dung) were collected before the starting of experiments. The sample's fresh weight (FW) was recorded followed by dry weight(DW) after samples were dried at 80° C for 72 hr's in hot air oven. The substrates moisture was calculated using the following formula.

$$\text{Substrates moisture(\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

- Desired moisture levels were maintained by adding additional volume of sterilized distilled water to the substrates as per treatment need.

Autoclaving, Inoculation and Incubation

The substrates with desired moisture were sterilized at 121.6° C (1.1 kg/cm²) for 20 minutes and after cooling, inoculated with 5 mm mycelia disc of actively growing culture of *T. harzianum* and cultural disc of *P. fluorescens* per flask. Inoculated flasks were incubated at 28± 2° C temperature. Visual observations of fungal growth in the inoculated flasks were made daily and mixed thoroughly by shaking the flask vigorously. The population dynamics of *T. harzianum* and *P. fluorescens* was determined by following serial dilution plate technique at the end of incubation period *i.e.* 10, 15 and 20 days after inoculation.

Determination of population dynamics

For monitoring the population dynamics, 1 gm substrate of each inoculated substrates & grown with respective bioagents was taken from each flasks and it was used for CFU_s count using PDA through dilution plate technique.

After each 5 days interval (10, 15 and 20 days after inoculation) *T. harzianum* and *P. fluorescens* population was determined by counting of colony forming units (CFU_s).

Colony Forming Units (CFU_s)

Serial dilution: Monitoring of population dynamics as colony forming units (CFU_s) of *T. harzianum* and *P. fluorescens* was done by serial dilution method. One gram of sample (substrate where *T. harzianum* and *P. fluorescens* was being grown) was suspended in 10 ml distilled water to make microbial suspension diluted 10 times, it was a 1: 10 conc. or 10⁻¹ dilution of original sample, *i.e.* the original sample was diluted to 1/10 th. Again, 1 ml of suspension from first tube get in to second tube contained 9 ml of sterile water; this suspension was used to make microbial concentration as 1 : 100 (10⁻²). Similarly it was prepared as 1: 1000 (10⁻³), 1: 10,000 (10⁻⁴), 1: 100,000 (10⁻⁵) up to (10⁻⁶) for fungi and (10⁻⁸) for bacteria on dilution of the original sample. Finally 1 ml of microbial suspension from last serial dilution *i.e.* (10⁻⁶) for *Trichoderma spp.* and (10⁻⁸) *Pseudomonas fluorescens* was added to sterile Petri dishes (triplicate in completely randomized manner) containing 30 ml of sterilized PDA medium, which was added with pinch of Streptomycin (to check bacterial contamination) when warm and molten. The Petri dishes were then incubated for 5 days at 28± 2° C.

C.F.U_s counting : The density of spores/ conidia in case of *T.harzianum* and bacterial also in case *P. fluorescens* (CFU_s) was measured in laboratory by plate dilution technique (Johnson and Curl, 1972).

$$\frac{\text{Colony (CFUs) count on an agar plate}}{\text{Total dilution of tube (used to make plate for colony count)}} \times \text{volume plated}$$

RESULT AND DISCUSSION

Screening of some new substrate for mass multiplication of *Trichoderma harzianum* and *Pseudomonas fluorescens* and determination of suitable moisture level

Screening of different substrate *viz.* wheat straw, wheat straw +rice husk, rice straw, sugarcane bagasse, sugarcane pressmud, bajra grains +wheat straw, sorghum grains + rice husk, chick pea flour + wheat straw, rotten cowdung + unrotten cowdung at five different moisture regime *i.e.* 0 percent, 10 percent, 30 percent, 50 percent and 70 percent was conducted for selection of mass suitable substrate and suitable moisture level. The results of experiment have been presented in tables.

Population dynamics of *T. harzianum* in different agriculture substrate at different moisture level

Population dynamics of *T. harzianum* at ten days after incubation: Data presented in the Table No.1 clearly indicate that without addition of any moisture, no CFU_s could be noticed in any substrate *viz.* wheat straw, wheat straw +rice husk, rice straw, sugarcane bagasse, sugarcane pressmud, bajra grain +wheat straw, sorghum grain + rice husk, chick pea flour + wheat straw, rotten cowdung + unrotten cowdung as no CFU_s could be retrieved from any substrate at 0% moisture.

At 10 percent level of moisture, *T.harzianum* could sustain its viability with the level of CFU_s, 10 × 10⁶ in sorghum grains + rice husk followed by (9.67 × 10⁶) bajra grains + wheat straw. The substrate, sugarcane pressmud (8.33 × 10⁶) followed by sugarcane bagasse (7.33 × 10⁶), rice straw (6.67 × 10⁶), chickpea flour+ wheat straw (6 × 10⁶), wheat straw (5.67 × 10⁶), wheat straw+ rice husk (5×10⁶) and rotten cow dung +

Table 1: Population dynamics of *Trichoderma harzianum* strain at different level of moisture in different agriculture substrate at ten days of inoculation

Treatments	Agriculture substrate	Colony forming unit of <i>T.harzianum</i> at different moisture regime (CFU _s ×10 ⁶)				
		0%	10 %	30%	50%	70%
T ₁	Wheat straw	0.00	5.67	6.33	6.33	8.67
T ₂	Wheat straw + Rice husk	0.00	5.00	6.00	6.33	8.00
T ₃	Rice straw	0.00	6.67	8.33	10.67	11.67
T ₄	Sugarcane bagasse	0.00	7.33	9.67	10.67	12.67
T ₅	Sugarcane pressmud	0.00	8.33	10.67	11.67	13.33
T ₆	Bajra grains + Wheat straw	0.00	9.67	10.67	12.67	14.67
T ₇	Sorghum grains + Rice husk	0.00	10.00	13.33	15.67	18.00
T ₈	Chickpea flour +Wheat straw	0.00	6.00	7.67	9.67	10.67
T ₉	Rotten cowdung +Unrotten cow dung	0.00	3.67	5.67	6.00	7.67
CD@5%level		1.366		1.349	0.910	1.355
SE(m)		0.460		0.454	0.306	0.456

unrotten cowdung (3.67×10^6), could support little low level of population dynamics.

With the addition of 30 percent moisture, sorghum grains + rice husk showed highest level of CFU_s (13.33×10^6) followed by bajra grains + wheat straw (10.67×10^6) both substrate were significantly different from each other with respect to supporting population dynamics of *T.harzianum*. Sugarcane pressmud and sugarcane bagasse resulted in (10.67×10^6) and (9.67×10^6) level of CFU_s but they were significantly lower than the CFU_s noticed in sorghum grains + rice husk and bajra grains + wheat straw. Some other substrates like rice straw (8.33×10^6), chickpea flour+ wheat straw (7.67×10^6) and wheat straw (6.33×10^6), resulted in quite lower level of CFU_s with a lowest by wheat straw+ rice husk (6×10^6), rotten cow dung + unrotten cowdung (5.67×10^6) respectively.

With the addition of 50 percent moisture, sorghum grains + rice husk showed highest level of CFU_s (15.67×10^6) followed by bajra grains + wheat straw(12.67×10^6) both substrate were significantly different from each other. Sugarcane pressmud and sugarcane bagasse resulted in (11.67×10^6) and (10.67×10^6) level of CFU_s but they were significantly lower than the CFU_s noticed in sorghum grains + rice husk

and bajra grains + wheat straw. Some other substrates like rice straw (10.67×10^6), chickpea flour + wheat straw (9.67×10^6), wheat straw (6.33×10^6), resulted in quite lower level of CFU_s with a lowest by wheat straw + rice husk (6.33×10^6), rotten cow dung + unrotten cow dung (6×10^6) respectively.

At 70 percent moisture, sorghum grains + rice husk showed highest level of CFU_s (18×10^6) followed by bajra grains + wheat straw(14.67×10^6) and sugarcane pressmud (13.33×10^6). However, the sugarcane bagasse (12.67×10^6), rice straw (11.67×10^6), chickpea flour + wheat straw (10.67×10^6), wheat straw (8.67×10^6), wheat straw + rice husk (8×10^6), rotten cow dung + unrotten cow dung (7.67×10^6) were found to be significantly lower than the population dynamics exhibited by sorghum grains + rice husk and bajra grains + wheat straw.

When all substrates were compared among them self based on the different moisture regimes i.e. 0 percent, 10 percent, 30 percent, 50 percent and 70 percent, it was noticed that highest CFU_s were noticed at 70 percent substrate moisture that was significantly higher than the population values exhibited at other moisture regimes. Substrate the sorghum grains + rice husk and bajra grains + wheat

Table 2: Population dynamics of *Trichoderma harzianum* strain at different level of moisture in different agriculture substrate at fifteen days of inoculation

Treatments	Agriculture substrate	Colony forming unit of <i>T.harzianum</i> at different moisture regime (CFU _s ×10 ⁶)				
		0%	10 %	30%	50%	70%
T ₁	Wheat straw	0.00	9.33	13.00	13.67	16.67
T ₂	Wheat straw + Rice husk	0.00	7.33	9.33	11.67	12.67
T ₃	Rice straw	0.00	11.33	14.33	17.33	20.33
T ₄	Sugarcane bagasse	0.00	11.33	14.67	18.67	23.00
T ₅	Sugarcane pressmud	0.00	11.67	15.33	20.33	26.67
T ₆	Bajra grains + Wheat straw	0.00	12.67	16.67	23.33	28.33
T ₇	Sorghum grains + Rice husk	0.00	15.67	19.67	25.67	32.00
T ₈	Chickpea flour +Wheat straw	0.00	11.00	13.67	13.33	16.67
T ₉	Rotten cowdung +Unrotten cow dung	0.00	6.67	8.67	11.67	11.67
CD@5% level		1.142		1.091	1.013	0.848
SE(m)		0.384		0.367	0.341	0.285

straw were found to be as in higher performer with regard to their capacity of supporting the level of population dynamics at all the moisture regimes and were significantly higher than rest of substrate.

Population dynamics at fifteen days after incubation: Data presented in the Table No. 2 clearly indicate that without addition of any moisture, no CFU_s could be noticed in any substrates viz. wheat straw, wheat straw + rice husk, rice straw, sugarcane bagasse, sugarcane pressmud, bajra grains + wheat straw, sorghum grains + rice husk, chick pea flour + wheat straw and rotten cow dung + unrotten cow dung.

With addition of 10 percent moisture, all the 9 substrate could sustain the viability of *T. harzianum*, out of all nine substrates. Sorghum grains + rice husk could support the viability of *T. harzianum* with a highest CFU_s of 15.67×10⁶ followed by 12.67 × 10⁶ in bajra grains + wheat straw. The sugarcane pressmud resulted in 11.67 × 10⁶ level of CFU_s which was significantly lower than sorghum grains + rice husk and bajra grains + wheat straw. There was 11.33 × 10⁶ no. of CFU_s noticed in sugarcane bagasse which was significantly less than sorghum grains + rice husk. The substrates rice straw (11.33 × 10⁶), chickpea flour + wheat straw (11.00 × 10⁶), and wheat straw (9.33×

10⁶) showed little less level of CFU_s followed by wheat straw + rice husk 7.33×10⁶ and rotten cow dung + unrotten cow dung (6.67 × 10⁶).

With addition of 30 percent moisture, the highest CFU_s were observed from sorghum grains + rice husk (19.67 × 10⁶) followed by bajra grains + wheat straw (16.67 × 10⁶) both substrate differed significantly with regard to no. of CFU_s at 30 percent moisture. The sugarcane pressmud resulted in 15.33 × 10⁶ CFU_s which was significantly lower than CFU_s noticed on sorghum grains + rice husk and bajra grains + wheat straw but significantly higher than rest of other substrate. In the sugarcane bagasse, there was 14.67 × 10⁶ level of CFU_s which was significantly lower than CFU_s noticed on sorghum grains + rice husk, bajra grains + wheat straw and sugarcane pressmud but quite higher than other substrate. Rest other substrates resulted comparatively low level of CFU_s i.e. rice straw (14.33 × 10⁶), chickpea flour + wheat straw (13.67 × 10⁶), wheat straw (13.00× 10⁶), wheat straw+ rice husk (9.33×10⁶) and rotten cow dung + unrotten cow dung (8.67 × 10⁶).

With the addition of 50 percent moisture, sorghum grains + rice husk (25.67 × 10⁶) followed by bajra grains + wheat straw (23.33× 10⁶) showed highest level of CFU_s with a significant difference from each other. Other

substrates i.e sugarcane pressmud (20.33×10^6), sugarcane bagasse (18.67×10^6), rice straw (17.33×10^6), chickpea flour + wheat straw (13.33×10^6), wheat straw (13.67×10^6), wheat straw + rice husk (11.67×10^6) and rotten cow dung + unrotten cowdung (11.67×10^6) also resulted in a considerably high level of CFU_s but they were significantly lower than Sorghum grains+ Rice husk and Bajra grains + Wheat straw.

With the addition of 70 percent moisture, sorghum grains+ rice husk showed highest level of CFU_s i.e. 32.00×10^6 followed by bajra grains + wheat straw (28.33×10^6) and sugarcane pressmud (26.67×10^6). However, these three substrates differed significantly from each other with respect to their capacity of supporting the no. of CFU_s at 70 percent moisture. The sugarcane bagasse resulted in 23.00×10^6 , rice straw exhibited 20.33×10^6 and chickpea flour + wheat straw exhibited 16.67×10^6 level of CFU_s but these values were significantly lower than the CFU_s noticed from sorghum grains + rice husk and bajra grains + wheat straw and also significantly different from each other. However, some other substrate i.e. wheat straw, wheat straw + rice husk and rotten cow dung + unrotten cow dung exhibited comparatively lower level of CFU_s i.e. (16.67×10^6), (12.67×10^6) and (11.67×10^6) respectively.

When all substrate were compared based on the different moisture regimes i.e. 0 percent, 10 percent, 30 percent, 50 percent and 70 percent, it was noticed that highest CFU_s were noticed at 70 percent substrate moisture that was significantly higher than the values at other moisture regimes. Substrates sorghum grains + rice husk and bajra grains + wheat straw were found to be higher performer with respect to their capacity of supporting CFU_s at all the moisture regimes and were significantly higher than rest of substrate.

Population dynamics at twenty days after incubation: Data presented in the Table No. 3 clearly indicate that without addition of any moisture, no CFU_s could be noticed in any substrates viz. wheat straw, wheat straw + rice husk, rice straw, sugarcane bagasse, sugarcane pressmud, bajra grains + wheat straw, sorghum grains + rice husk, chick pea flour + wheat straw and rotten cow dung + unrotten cow dung.

At 10 percent moisture, sorghum grains + rice husk showed highest level of CFU_s (28.67×10^6) followed by bajra grains + wheat straw (26.33×10^6), which were significantly different from each other. One substrate of viz. sugarcane pressmud exhibited (23.00×10^6), level of CFU_s which was significantly, higher than other substrate but it was significantly

Table 3: Population dynamics of *Trichoderma harzianum* strain at different level of moisture in different agriculture substrate at twenty days of inoculation

Treatments	Agriculture substrate	Colony forming unit of <i>T.harzianum</i> at different moisture regime (CFU $\times 10^6$)				
		0%	10 %	30%	50%	70%
T ₁	Wheat straw	0.00	14.33	17.33	20.33	23.00
T ₂	Wheat straw + Rice husk	0.00	11.33	14.67	17.67	20.33
T ₃	Rice straw	0.00	18.67	22.67	26.33	29.33
T ₄	Sugarcane bagasse	0.00	20.33	23.33	27.67	30.67
T ₅	Sugarcane pressmud	0.00	23.00	26.67	31.67	39.33
T ₆	Bajra grains + Wheat straw	0.00	26.33	30.00	36.33	43.67
T ₇	Sorghum grains + Rice husk	0.00	28.67	36.33	45.00	58.67
T ₈	Chickpea flour +Wheat straw	0.00	15.67	20.33	24.67	27.00
T ₉	Rotten cowdung +Unrotten cow dung	0.00	9.00	12.67	16.67	18.67
CD@5%level			1.091	0.837	0.807	0.841
SE(m)			0.367	0.282	0.2720	0.283

lower than the CFU_s noticed on sorghum grains + rice husk and and bajra grains + wheat straw. Rest of substrate viz. sugarcane bagasse, rice straw, chickpea flour + wheat straw, wheat straw, wheat straw + rice husk and rotten cow dung + unrotten cow dung exhibited (20.33 × 10⁶) (18.67 × 10⁶), (15.67 × 10⁶), (14.33 × 10⁶), (11.33 × 10⁶) and (9.00 × 10⁶) level of CFU_s and obviously were lower than the population dynamics noticed at earlier described three substrates.

With the addition of 30 percent moisture, the highest level of CFU_s (36.33 × 10⁶) and (30.00 × 10⁶) were noticed in two substrate i.e. sorghum grains + rice husk and bajra grains + wheat straw. The substrate, sugarcane pressmud exhibited (26.67 × 10⁶), level of CFU_s followed by sugarcane bagasse (23.33 × 10⁶), rice straw (22.67 × 10⁶) and chickpea flour + wheat straw (20.33 × 10⁶) but all these substrate were significantly lower than the CFU_s noticed on sorghum grains + rice husk and bajra grains + wheat straw and also significantly different than each other. The rest three substrate resulted in least level of CFU_s i.e. wheat straw (17.33 × 10⁶), wheat straw + rice husk (14.67 × 10⁶) and rotten cow dung + unrotten cow dung (12.67 × 10⁶) and obviously were poor performer.

At 50 percent level of moisture, the maximum level of CFU_s was recorded in sorghum grains + rice husk (45.00 × 10⁶) and bajra grains + wheat straw (36.33 × 10⁶) which were significantly different from each other. Other substrates like, sugarcane pressmud (31.67 × 10⁶), sugarcane bagasse (27.67 × 10⁶) and rice straw (26.33 × 10⁶) also resulted in a quite high level of CFU_s but were significantly lower than the CFU_s noticed on sorghum grains + rice husk & bajra grains + wheat straw but significantly different among themselves. Some other substrates like chickpea flour + wheat straw (24.67 × 10⁶) and wheat straw (20.33 × 10⁶) resulted in comparatively low level of CFU_s. The rest two substrate namely, wheat straw + rice husk (17.67 × 10⁶) and rotten cow

dung + unrotten cow dung (16.67 × 10⁶) resulted in lowest level of CFU_s hence considered to be poorer one.

With the addition of 70 percent moisture, sorghum grains + rice husk showed maximum level of CFU_s (58.67 × 10⁶) followed by bajra grains + wheat straw (43.67 × 10⁶), sugarcane pressmud (39.33 × 10⁶) and sugarcane bagasse (30.67 × 10⁶), however all these substrate differed significantly with respect to level of CFU_s. The other substrates like rice straw exhibited 29.33 × 10⁶ CFU_s, chickpea flour + wheat straw exhibited 27.00 × 10⁶ and wheat straw gave 23.00 × 10⁶ level of CFU_s but these substrate were significantly lower than the CFU_s noticed on sorghum grains + rice husk, bajra grains + wheat straw and sugarcane pressmud. The rest two substrate viz. wheat straw + rice husk (20.33 × 10⁶) and rotten cow dung + unrotten cow dung (18.67 × 10⁶) were minimum level of CFU_s.

When all substrate were compared based on the different moisture regimes i.e. 0 percent, 10 percent, 30 percent, 50 percent and 70 percent, it was noticed that highest CFU_s among all substrates were noticed at 70 percent substrate moisture that was significantly higher than the values at other moisture regimes. Sorghum grains + rice husk and bajra grains + wheat straw were found to be highest performer with respect to their CFU_s level at all the moisture regimes and were significantly higher than rest of substrate.

Population dynamics of *P. fluorescens* in different agriculture substrate at different moisture level.

Population dynamics of P. fluorescens at ten days after incubation: Data presented in the Table No. 4 clearly indicate that without addition of any moisture, no CFU_s could be noticed in any substrate viz. wheat straw, wheat straw + rice husk, rice straw, sugarcane bagasse, sugarcane pressmud, bajra grains + wheat straw, sorghum grains + rice husk, chick

Table 4: Population dynamics of *Pseudomonas fluorescence* strain at different level of moisture in different agriculture substrate at ten days of inoculation

Treatments	Agriculture substrate	Colony forming unit of <i>P. fluorescence</i> at different moisture regime (CFU _s ×10 ⁶)				
		0%	10 %	30%	50%	70%
T ₁	Wheat straw	0.00	23.33	24.67	26.33	29.33
T ₂	Wheat straw + Rice husk	0.00	27.33	26.67	30.67	32.67
T ₃	Rice straw	0.00	31.33	33.33	37.00	42.33
T ₄	Sugarcane bagasse	0.00	15.67	17.00	19.67	19.33
T ₅	Sugarcane pressmud	0.00	22.33	23.00	24.33	28.67
T ₆	Bajra grains + Wheat straw	0.00	15.00	16.67	17.67	18.33
T ₇	Sorghum grains + Rice husk	0.00	19.00	22.00	23.67	28.33
T ₈	Chickpea flour +Wheat straw	0.00	34.33	40.00	41.00	43.67
T ₉	Rotten cowdung +Unrotten cow dung	0.00	13.00	14.33	15.67	17.67
CD@5%level			1.089	1.101	1.181	1.226
SE(m)			0.367	0.371	0.398	0.413

pea flour + wheat straw and rotten cow dung + unrotten cow dung.

At 10 percent level of moisture, *Pseudomonas fluorescens* could sustain its viability with the level of CFU_s 10 × 10⁶ in chickpea flour + wheat straw (34.33 × 10⁶) followed by rice straw (31.33 × 10⁶). Some other substrates like wheat straw + rice husk (27.33×10⁶) followed by wheat straw (23.33 × 10⁶), sugarcane pressmud (22.33 × 10⁶), sorghum grains + rice husk (19.00× 10⁶) and sugarcane bagasse (15.67×10⁶) also resulted in quite high level of CFU_s with a lowest by bajra grains + wheat straw (15.00× 10⁶) and rotten cow dung + unrotten cow dung (13.00 × 10⁶)

With the addition of 30 percent moisture, chickpea flour + wheat straw showed highest level of CFU_s (40.00 × 10⁶) followed by rice straw (33.33 × 10⁶) both these substrates were significantly different from each other . Wheat straw + rice husk and wheat straw resulted in (26.67 × 10⁶) and (24.67 × 10⁶) level of CFU_s but they were significantly lower than the CFU_s noticed on chickpea flour + wheat straw and rice straw. Some other substrates like sugarcane pressmud (23.00 × 10⁶), sorghum grains + rice husk (22.00× 10⁶)and sugarcane bagasse (17.00× 10⁶), resulted in quite lower level of CFU_s with a lowest by bajra grains + wheat straw (16.67×10⁶) and rotten cow dung + unrotten cow dung (14.33 × 10⁶).

At 50 percent level of moisture, chickpea flour + wheat straw showed highest level of CFU_s (41.00× 10⁶) followed by rice straw (37.00× 10⁶) both substrate were significantly different from each other . Wheat straw + rice husk and wheat straw resulted in 30.67 × 10⁶ and 26.33 × 10⁶ level of CFU_s but they were significantly lower than the CFU_s noticed on chickpea flour + wheat straw and rice straw. Some other substrates like sugarcane pressmud (24.33 × 10⁶), sorghum grains + rice husk (23.67× 10⁶) and sugarcane bagasse (19.67× 10⁶), resulted in quite lower level of CFU_s with a lowest by bajra grains + wheat straw (17.67×10⁶) and rotten cow dung + unrotten cow dung (15.67 × 10⁶).

With addition of 70 percent moisture, chickpea flour + wheat straw showed highest level of CFU_s (43.67× 10⁶) followed by rice straw (42.33× 10⁶) and wheat straw + rice husk (32.67× 10⁶). However, the substrates wheat straw (29.33 ×10⁶), sugarcane pressmud (28.67 × 10⁶), sorghum grains + rice husk (28.33× 10⁶), sugarcane bagasse (19.33× 10⁶), bajra grains + wheat straw (18.33×10⁶) and rotten cow dung + unrotten cow dung (17.67 × 10⁶) were found to be significantly lower than the CFU_s noticed on chickpea flour + wheat straw and rice straw. When all substrates were compared based on their performance

at different moisture regimes i.e. 0 percent, 10 percent, 30 percent, 50 percent and 70 percent, it was noticed that highest CFU_s were noticed at 70 percent substrate level of moisture which that was significantly higher than the values at other moisture regimes. Substrate i.e. chickpea flour + wheat straw and rice straw resulted in higher performance with regard to their CFU_s level at all the moisture regimes and were significantly higher than rest of substrate.

Population dynamics at fifteen days after incubation: Data presented in the Table No. 5 clearly indicate that without addition of any moisture, no CFU_s could be noticed in any substrates viz. wheat straw, wheat straw + rice husk, rice straw, sugarcane bagasse, sugarcane pressmud, bajra grains + wheat straw, sorghum grains + rice husk, chick pea flour + wheat straw and rotten cow dung + unrotten cow dung as no CFU_s could be retrieved from any substrate at 0% moisture.

With the addition of 10 percent moisture, all the 9 substrate could sustain their viability, out of these nine substrates, chick pea flour + wheat straw resulted in highest CFU_s, 40.00 × 10⁶ followed by 39.00 × 10⁶ in rice straw. The wheat straw + rice husk (31.33 × 10⁶) and wheat straw resulted in 28.33 × 10⁶ CFU_s which was significantly lower than the CFU_s noticed on chick pea flour + wheat straw and rice straw. There was 26.67 × 10⁶ no. of CFU_s in

sugarcane pressmud which was significantly less than chick pea flour + wheat straw. Some other substrates like sorghum grains + rice husk (26.33 × 10⁶) and sugarcane bagasse (17.33 × 10⁶) resulted in little lower level of CFU_s with a lowest by bajra grains + wheat straw (16.67 × 10⁶) and rotten cow dung + unrotten cow dung (16.00 × 10⁶).

At 30 percent of moisture, the highest CFU_s were resulted from chickpea flour + wheat straw (44.33 × 10⁶) followed by rice straw (42.00 × 10⁶) both substrate significantly differed with regard to no. of CFU_s at 30 percent moisture. The wheat straw + rice husk resulted in 34.00 × 10⁶ CFU_s which was significantly lower than the CFU_s noticed in chickpea flour + wheat straw and rice straw but significantly higher than rest of other substrate. Wheat straw resulted in 32.00 × 10⁶ level of CFU_s which was significantly lower than chickpea flour + wheat straw, rice straw and wheat straw + rice husk but quite higher than other substrate. Rest other substrate showed comparatively low level of CFU_s i.e. sugarcane pressmud (29.67 × 10⁶), sorghum grains + rice husk (26.67 × 10⁶), sugarcane bagasse (22.00 × 10⁶), bajra grains + wheat straw (21.67 × 10⁶) and rotten cow dung + unrotten cow dung (18.00 × 10⁶) which were also quite considerable with regards to the level of their population dynamics they exhibited.

Table 5: Population dynamics of *Pseudomonas fluorescense* strain at different level of moisture in different agriculture substrate at fifteen days of inoculation

Treatments	Agriculture substrate	Colony forming unit of <i>P. fluorescense</i> at different moisture regime (CFU × 10 ⁶)				
		0%	10 %	30%	50%	70%
T ₁	Wheat straw	0.00	28.33	32.00	34.67	36.67
T ₂	Wheat straw + Rice husk	0.00	31.33	34.00	35.33	40.33
T ₃	Rice straw	0.00	39.00	42.00	42.33	47.67
T ₄	Sugarcane bagasse	0.00	17.33	22.00	24.33	24.33
T ₅	Sugarcane pressmud	0.00	26.67	29.67	34.33	36.67
T ₆	Bajra grains + Wheat straw	0.00	16.67	21.67	23.33	24.20
T ₇	Sorghum grains + Rice husk	0.00	26.33	26.67	30.33	33.00
T ₈	Chickpea flour + Wheat straw	0.00	40.00	44.33	49.33	54.00
T ₉	Rotten cowdung + Unrotten cow dung	0.00	16.00	18.00	20.67	23.67
CD@5% level			1.214	1.170	1.317	1.599
SE(m)			0.409	0.394	0.443	0.538

With the addition of 50 percent moisture, chickpea flour + wheat straw (49.33×10^6) followed by rice straw (42.33×10^6) showed highest level of CFU_s both substrate were significantly different from each other. Other substrate *i.e.* wheat straw + rice husk (35.33×10^6), wheat straw (34.67×10^6), sugarcane pressmud (34.33×10^6), sorghum grains + rice husk (30.33×10^6), sugarcane bagasse (24.33×10^6), bajra grains + wheat straw (23.33×10^6) and rotten cow dung + unrotten cow dung (20.67×10^6) also resulted in a considerably high level of CFU_s but they were significantly lower than chickpea flour + wheat straw and rice straw.

At 70 percent level of moisture, chick pea flour + wheat straw showed highest level of CFU_s *i.e.* 54.00×10^6 followed by rice straw (47.67×10^6) and wheat straw + rice husk (40.33×10^6). However, these three substrate differed significantly from each other with respect to the no. of CFU_s at 70 percent moisture. The wheat straw resulted in 36.67×10^6 , sugarcane pressmud 36.67×10^6 and sorghum grains + rice husk 33.00×10^6 no. of CFU_s but these values were significantly lower than CFU_s retrieved from chick pea flour + wheat straw and rice straw and also significantly different from each other. However, some other substrate *i.e.* sugarcane bagasse, bajra grains + wheat straw and rotten cow dung + unrotten cow dung exhibited 24.33×10^6 , 24.20×10^6 and $23.67 \times$

10^6 level of population which were also quite considerable.

When all substrate were compared based on the different moisture regimes *i.e.* 0 percent, 10 percent, 30 percent, 50 percent and 70 percent, it was noticed that highest CFU_s were noticed at 70 percent moisture which was significantly higher than the values at other moisture regimes. Other substrates *i.e.* chickpea flour + wheat straw and rice straw were also found to be best performer with regard to their CFU_s values at all the moisture regimes tested and were significantly higher than rest other substrate.

Population dynamics at twenty days after incubation: Data presented in the Table No. 6 clearly indicate that without addition of any moisture, no CFU_s could be noticed in any substrate.

At 10 percent moisture, chick pea flour + wheat straw showed highest level of CFU_s *i.e.* (53.00×10^6) followed by rice straw (43.67×10^6), which were significantly different from each other. Other substrates *viz.* wheat straw + rice husk exhibited 36.67×10^6 , level of CFU_s which was significantly different from each substrate but it was significantly lower than the CFU_s noticed on chickpea flour + wheat straw and rice straw. Remaining substrates *viz.* wheat straw, sugarcane pressmud, sorghum grains

Table 6: Population dynamics of *Pseudomonas fluorescense* strain at different level of moisture in different agriculture substrate at twenty days of inoculation

Treatments	Agriculture substrate	Colony forming unit of <i>P. fluorescense</i> at different moisture regime (CFU $\times 10^8$)				
		0%	10 %	30%	50%	70%
T ₁	Wheat straw	0.00	32.67	36.33	37.67	41.67
T ₂	Wheat straw + Rice husk	0.00	36.67	41.33	42.33	45.00
T ₃	Rice straw	0.00	43.67	44.33	47.67	52.33
T ₄	Sugarcane bagasse	0.00	22.00	27.00	25.33	29.33
T ₅	Sugarcane pressmud	0.00	32.33	36.00	37.67	41.00
T ₆	Bajra grains + Wheat straw	0.00	20.67	22.67	24.33	28.00
T ₇	Sorghum grains + Rice husk	0.00	31.67	33.67	35.67	40.33
T ₈	Chickpea flour +Wheat straw	0.00	53.00	54.67	57.00	59.67
T ₉	Rotten cow dung +Unrotten cow dung	0.00	18.33	22.33	24.00	25.00
CD@5%level			1.451	1.369	1.337	1.222
SE(m)			0.489	0.461	0.450	0.411

+ rice husk, sugarcane bagasse, bajra grains + wheat straw and rotten cow dung + unrotten cow dung resulted in 32.67×10^6 , 32.33×10^6 , 31.67×10^6 , 22.00×10^6 , 20.67×10^6 and 18.33×10^6 no. of CFU_s respectively.

With the addition of 30 percent moisture, the highest level of CFU_s (54.67×10^6) and (44.33×10^6) were recorded in two substrate *i.e.* chickpea flour + wheat straw and rice straw. Other substrates *viz.* wheat straw + rice husk exhibited (41.33×10^6), level of CFU_s followed by wheat straw (36.33×10^6), sugarcane pressmud (36.00×10^6) and sorghum grains + rice husk (33.67×10^6) but these values were significantly lower than the CFU_s noticed in chickpea flour + wheat straw and rice straw. The rest three substrate resulted in least level of CFU_s *i.e.* sugarcane bagasse (27.00×10^6), bajra grains + wheat straw (22.67×10^6) and rotten cow dung + unrotten cow dung (22.33×10^6) obviously were poor performer.

At 50 percent moisture, the maximum level of CFU_s were recorded from chickpea flour + wheat straw (57.00×10^6) and rice straw (47.67×10^6) which were significantly different from each other. Other substrate like, wheat straw + rice husk (42.33×10^6), wheat straw (37.67×10^6) and sugarcane pressmud (37.67×10^6) also resulted in a quite high level of CFU_s but they were significantly lower than the CFU_s noticed in chickpea flour + wheat straw & rice straw significantly different among themselves. Some other substrate like sorghum grains + rice husk (35.67×10^6) and sugarcane bagasse (25.33×10^6) resulted in comparatively low level of CFU_s. The rest two substrate namely, bajra grains + wheat straw (24.33×10^6) and rotten cow dung + unrotten cow dung (24.00×10^6) resulted in lowest level of CFU_s hence considered to be poorer one.

With the addition of 70 percent moisture, chick pea flour + wheat straw showed maximum level of CFU_s (59.67×10^6) followed by rice straw (52.33×10^6), wheat straw + rice husk (45.00×10^6) and wheat straw ($41.67 \times$

10^6) however all these substrate differed significantly with respect to level of CFU_s. The substrate like sugarcane pressmud exhibited 41.00×10^6 , sorghum grains + rice husk exhibited 40.33×10^6 and sugarcane bagasse gave 29.33×10^6 no. of CFU_s but these substrate were significantly lower than the CFU_s noticed in chickpea flour + wheat straw, rice straw and wheat straw + rice husk. The rest two substrate *viz.* bajra grains + wheat straw (28.00×10^6) and rotten cow dung + unrotten cow dung (25.00×10^6) were resulted in minimum level of CFU_s.

When all substrate were compared for their performance based on the different moisture regimes *i.e.* 0 percent, 10 percent, 30 percent, 50 percent and 70 percent, it was noticed that highest CFU_s of all substrate were noticed at 70 percent substrate moisture which was significantly higher than the values at other moisture regimes. Substrate *i.e.* chickpea flour + wheat straw and rice straw were noticed to be higher performance with regard to their CFU_s level at all the moisture regimes and were significantly superior than rest of substrate.

These findings suggested that 70 percent additional moisture in the substrate of sorghum grains + rice husk and bajra grains + wheat straw is most suitable for highest level of population dynamics of *T.harzianum*. For *P.fluorescens* two substrate *i.e.* chickpea flour + wheat straw and rice straw were found to be best performer.

Tiwari and Bhanu (2004)- conducted an experiment for management of agro-industrial wastes and selection of a suitable cheaper substrate for bio-pesticide development, three categories of agro-industrial wastes, *viz.* (1) Cellulose wastes (2) Organic manures and (3) cereals brans were evaluated for growth response and conidial yield of bio-control agent *Trichoderma harzianum*. All the substrates supported fairly good growth and sporulation of bio-agent, but conidial counts were maximum in rice bran at 15 d followed by paddy straw and wheat straw at 20 d.

Supplementation of all substrates with chickpea flour as organic nitrogen supplement enhanced the conidial yield, maximum increase in spore count was recorded in the case of paper waste. The optimum level of chickpea flour supplementation for paper waste is recorded as 12 %, thereby showing maximum conidiospore production (17.07×10^8 cfu/g) in 20 at, a further increment in cfu count was recorded upon its supplementation with rice bran. Paper waste based mixed substrate combination containing paper waste (80%), rice bran (8%) and chickpea flour (12%) show considerably higher conidial yield (11.73×10^9 cfu/g) after 10 d incubation period, exploring the possibility of utilizing it for mass multiplication and large scale inoculum production of eco-friendly bio-control agent for suitable environment.

Yadav (2012)- isolated two species of *Trichoderma* viz. *T. viride* and *T. harzianum* from soil and their *in-vitro* antagonistic activities were demonstrated against plant pathogenic fungi *Colletotrichum gloeosporioides* and *Fusarium oxysporum* by dual culture method. Various agricultural residues and by products such as rice husk, saw dust, maize husk and wheat bran were evaluated for mass production of *Trichoderma viride* and *Trichoderma harzianum*. Among the substrates used, maize husk was found to support maximum spore production for *Trichoderma viride* (2.0×10^8 per gram substrate) and 1.2×10^8 per gram substrate for *Trichoderma harzianum* as compared with other substrate like wheat bran, rice husk and saw dust respectively.

CONCLUSION

Sorghum grains + rice husk and chickpea flour + wheat straw, both substrate can be used for

mass multiplication of *T. harzianum* and *P. fluorescens*, even at 30 % additional moisture. However 70 percent moisture to these substrate to be most appropriate.

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REFERENCES

- Bandopadhyay, S., Josh, S. and Dutta, S. (2003). Effect of different P^H and temperature level on growth and sporulation of *Trichoderma*. *Environment & Ecology*, **21**: 770-773.
- Elad, Y., Chet, I. and Kalan, J. (1980). *Trichoderma harzianum*: a biocontrol agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. *Phytopathology*. **70**: 719-25.
- Fravel, D.R. (2005). Commercialization and implementation of biocontrol. *Annual Review of Phytopathology* **43**: 337-59.
- Patibandha, A. K., Upadhyay, J. P. and Mukhopadhyay, A. N. (2003). Efficacy of *Trichoderma harzianum* Rifai alone or in combination with fungicides against sclerotium wilt of groundnut. *Journal of Biological Control* **17** : 57-67.
- Reddy, T. K. R. and Khowles, R. (1965). The fungal flora of aboreal forest raw humus. *Canadian Journal of Microbiology*, **1**: 837-843.
- Tiwari, L. and Bhanu, C. (2004). Evaluation of agro-industrial wastes for conidial based inoculums production of bio-control agent: *Trichoderma harzianum*. *Journal of Scientific & Industrial Research*; **63**: 807-812.
- Yadav, L. S. (2012). Antagonistic activity of *Trichoderma* spp. and evaluation of various agrowastes for mass production. *Indian Journal of Plant Sciences*; **1** (1) : 109-112.