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Standardization of inoculum for mass multiplication of *Trichoderma*

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Mass multiplication of biocontrol agent is necessary for biological control of plant pathogens. The present investigation was undertaken to standardize the inoculums for mass multiplication of *Trichoderma*. The unit inoculum of wheat medium @ 20 g, 30 g, 40 g, 50 g and 60 g per 10 kg of oilcake were inoculated and incubated in BOD at 28±1°C. The results revealed that there was gradual increase in cfu up to 1st week, then the cfu production gradually decreased in all the treatments except in treatments Tr_2 (30 g inoculum/10 kg oilcake), Tr_3 (40 g inoculum/10 kg oilcake) and Tr_4 (50 g inoculum/10 kg oilcake) where after 3rd week cfu production increased and then decreased again. Among all the treatments maximum cfu production was recorded in 4th week except in treatments Tr, (20 g inoculum/10 kg oilcake) and Tr_5 (60 g inoculum/10 kg oilcake). From the results it can be concluded that wheat seeds used as substrate for the production of mass culture of *T.harzianum,* may be used for distribution to the farmers after 5-6 weeks of inoculation.

Key words: Mass multiplication, biocontrol agent, *Trichoderma*

INTRODUCTION

The genus *Trichoderma* is known to include several potentially promising hyperparasites/ antibiotic producers that have promise against a large number of soil borne plant pathogens (Chet *et al.*, 1979; Lewis and Papavizas, 1991; Mohanty *et al.*, 2000; D'Souza *et al,* 2001; Roy, 2001). For the biological control of plant pathogens on a field scale, it is necessary to produce biocontrol agents in a large scale in the form of spores or other propagules. The population of biocontrol agents in a unit substrate should be applied in a higher amount preferably above 10^6 populations, for the control of plant pathogens as well as to combat with the other microorganisms (Chet *et al.,* 1979). The present investigation has been undertaken to standardize the inoculum for mass multiplication of *Trichoderma*.

MATERIALS AND METHODS

Isolation of Trichoderma from soil

Isolation of biocontrol agents was made from the soil samples collected from BCKV experimental baroj at Kalyani. Soil samples were collected at random from a depth of 15 cm, after cleaning the soil surface. The samples were then mixed together, kept moist in plastic bags, labeled and stored in refrigerator. Ten grams of air dried soil sample was sieved through 2 mm mesh and placed in 100 ml sterile distilled water and shaken vigorously for proper mixing. Further dilutions were maintained under constant shaking. One ml of each dilution was pipetted on to sterile Petriplates in replication after constant vigorous shaking of the solution. After this about 15-20 ml of TSM (modified) was poured in each plate at 40 - 45 °C aseptically. For uniform distribution and mixing of medium and soil solution, the plates were rotated in both directions i.e. clockwise and anticlockwise. The plates were incubated at 28 ± 1 °C for 4 days, keeping the plates under fluorescent light on 3rd day. After 4 days the typical greenish colonies developed were subcultured and morphological characters were observed under light microscope. Those that appeared to be *Trichoderma* sp. were maintained on PDA medium and stored in a refrigerator at 5 °C. Sub-culturing was done at 15 days interval.

Mass multiplication of Trichoderma

Unbroken, wheat seeds un-treated with fungicides or insecticides were boiled with an equal volume of water till the water dries out. The grain should become soft but it should not get split. If there was excess water it was drained off and mixed thoroughly with calcium carbonate @ 8% by grain weight. The mixture poured in conical flask and sterilized two times at 121°C for 30 minutes. Then the mixture was inoculated with 3 days old mycelial disc of *Trichoderma* sp. which was previously isolated in TSM medium from the soil samples collected from BCKV experimental baroj as mentioned earlier. The flasks were then incubated in a BOD incubator at 28±1° for 30 days. After 30 days of inoculation, the culture of *Trichoderma* grown in wheat medium was used to inoculate the oilcake medium. The moistened de-oiled mustard oil cake packed in double polypropylene bag was double sterilized in autoclave at 121°C for 30 minutes. Inoculum on wheat medium @ 20 g, 30 g, 40 g, 50 g and 60 g were inoculated to propylene bags containing 10 kg sterilized mustard oilcake.

Population (cfu) of *Trichoderma* sp. was recorded at an interval of one week up to 8 weeks of inoculation, on oilcake by growing the culture in TSM medium. The data obtained were subjected to regression analysis.

RESULTS AND DISCUSSION

The results (Table 1) revealed that highest population at "0" day was recorded in treatment where 60 g unit of inoculum was used whereas lowest population was recorded in treatment where 20 g unit of inoculum. Gradual increase in cfu production up to 3 weeks were recorded in all treatments except where 30 g and 50 g unit of inoculum were used. Again, increase in cfu was recorded after 4 weeks in the above two treatments (30 g and 50 g unit of inoculum 10 kg oilcake) . After that the cfu production decreased except in treatment where 40 g unit was inoculated per 10 kg oilcake which recorded increase up to 5 weeks. From 5 weeks onward, there was decrease in all the treatments.

It is known that *Trichoderma,* a good saprophyte, acts as hyperparasite at relatively higher populations

Table 1: Colony forming unit / gm of soil of *Trichoderma harzianum* as a function of time in mustard oil cake after growing in wheat grain and survival time of *Trichoderma harzianum* in mustard oil cake up to threshold level

Treatments	Weeks									Regression equation	R^2	Survival time of *T. harzianumin* in MOC up to threshold level*
	0	1	2	3	4	5	6	7	8			
20 g/10 kg mustard oilcakes	5×10^6	4×10^7 (700.0)	6×10^7 (50.0)	8×10^7 (33.33)	6×10^7 (-33.33)	3×10^7 (-50.0)	2×10^6 (-93.33)	1.33×10^6 (-33.5)	1×10^6 (-24.81)	Y = 509.04-49.66 X	0.20	56
30 g/10 kg mustard oilcakes	8×10^6	8×10^7 (900.0)	8×10^7 (00.0)	7×10^7 (-12.5)	1.2×10^8 (71.42)	9×10^7 (-25.0)	5×10^6 (-94.44)	1.67×10^6 (-66.6)	6×10^5 (-64.07)	Y = 768.9-65.76 X	0.15	55
40 g/10 kg mustard oilcakes	8×10^6	7×10^7 (775.0)	8×10^7 (14.28)	9×10^7 (12.5)	1×10^8 (11.11)	1.2×10^8 (20.0)	3×10^7 (-75.0)	8×10^6 (-73.33)	6×10^5 (-97.91)	Y = 753.96-47.88 X	0.08	55
50 g/10 kg mustard oilcakes	9×10^6	9×10^7 (900.0)	1×10^8 (11.11)	7×10^7 (-30.0)	1.4×10^8 (100.0)	1×10^8 (-28.5)	4×10^7 (-60.0)	3×10^6 (-92.5)	9.33×10^5 (-68.9)	Y = 856.88-63.87 X	0.12	55
60 g/10 kg mustard oilcakes	2×10^6	9×10^7 (375.0)	1×10^8 (11.11)	2×10^8 (100.0)	1.6×10^8 (-20.0)	1.4×10^8 (-57.14)	6×10^7 (-57.14)	2×10^7 (-366.66)	7×10^5 (-96.5)	Y = 1163.35-71.2 X	0.08	55

Figure in parentheses are the per cent increase / decrease in cfu production *Data presented are time in days required to bring down the cfu/g to 1 $x10^6$

and the threshold has been fixed at around 1 x 10^6 cfu/g soil (Adams, 1990; Baker and Dickman, 1993). Survival of the isolates in mustard oil cake as a function of time was plotted as a function of time with populations transformed to log. Following linear regression, the time required for population to reach the threshold level 1 x10^6 cfu/g MOC was determined and it was recorded that the above population reached in 55-56 days in all the treatments (Table 1).

The results obtained confirmed the findings of other scientists. Sanyal *et al.,* (2003) studied the survival of *Trichoderma* in alginate prills and showed that survivability of *Trichoderma* was higher (60-77 days) when Ca-G was used than when $CaCl_3$ (32-45 days) was used as gellant. From the results, it can be concluded that wheat seed used as substrate for the production of mass culture of *Trichoderma* may be used for distribution to the farmers after 56 days of inoculation of minimum 20 gm of inoculum of *Trichoderma* per 10 kg oilcake. The cfu production in such treatment is 1x10 cfu/g which is recommended for application of bioagent under field condition in most of the cultivable crops for the control of soil borne diseases.

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